EFFECT OF MECHANICAL PRETREATMENT ON SOLUBILIZATION AND BIOMETHANATION OF FOOD WASTE

By

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To my parents and Kim, thank you for all your support and encouragement

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The current disposal of food waste in landfills is an unsustainable practice that causes many environmental, social, and economic problems. Diverting food waste from landfills for anaerobic digestion presents the opportunity for three positive outcomes: sustainable waste utilization, production of renewable bioenergy, and recovery of biofertilizer. Currently, food waste digestion does not exist at the commercial scale outside of co-digestion with manure or municipal sludge. The anaerobic digestion of food waste is considered to be limited by the rate of hydrolysis, which impedes the use of food waste as a feedstock in high-rate digestion. Food waste pretreatment has been examined to increase the hydrolytic rate of food waste. Current literature reports pretreatment methods that are not economically feasible for commercial-scale digestion. The present study examined the use of practical mechanical pretreatment (food disposer and meat grinder) for increasing the solubilization and biomethanation of food waste.

Three solubilization assays were conducted to compare the solubilization kinetics of intact food waste to those of pretreated food waste. The three assays measured 1) the release of endogenous soluble organic material, 2) enzymatic hydrolysis with excess commercial hydrolytic enzymes, and 3) enzymatic hydrolysis using a microbial inoculum. In all three assays, pretreated food waste exhibited significantly higher solubilization kinetics than intact

food waste. Pretreatment of food waste released the full complement of soluble organic material (25% solubilization) immediately, while intact food waste was 4.1% solubilized initially and 21% solubilized at 8 h through the leaching of endogenous soluble organics at a 1st order rate (k) of 0.36 h^{-1}. With excess commercial hydrolytic enzymes, pretreated and intact food waste was 60% and 49%, respectively, solubilized in 8h with enzymatic hydrolysis rates of 0.84 to 1.19 h^{-1} and 0.25 h^{-1}, respectively. When incubated with a microbial inoculum, pretreated and intact food waste was 52% and 37% solubilized in 24 h, which indicated that, for pretreated food waste, a microbial inoculum is nearly as effective in 24 h as excess commercial enzymes in 8h. In an active anaerobic digester, the microbial hydrolysis rate would be even greater than in the assay. The results showed that mechanical pretreatment increased the solubilization kinetics of food waste, which increased the available substrate for biomethanation.

Two biochemical methane potential assays were performed on intact and pretreated (meat grinder with 0.5 cm plate openings) food waste. The first assay was loaded at 3.5 g COD/L, while the second assay was loaded at 2 g COD/L. The first assay at the moderate loading rate showed slower methanogenic kinetics (k=0.12 d^{-1}) than the second assay at the reduced loading rate (k=0.20 d^{-1}). Pretreated food waste at the moderate loading rate showed a lower cumulative methane yield than the intact food wastes in either assay (282 and 320 mL/g COD, respectively). These results were due to acidification at the moderate loading rate, which was exacerbated through pretreatment. The experiments show that in the BMP assay, methanogenesis and not hydrolysis was the rate-limiting step, and at moderate loading rates, increased solubilization can inhibit methanogenesis. Therefore, using a high-rate digester, such as a fixed-film reactor with retained methanogenic biomass, is necessary to accommodate increased solubilization and acidogenesis of pretreated food waste.

CHAPTER 1
INTRODUCTION

The current disposal of food waste is a global problem that is garnering increased public attention. Food waste represents a significant proportion of our municipal solid waste (MSW). The United States Environmental Protection Agency (U.S. EPA) estimates that in 2008, the U.S. generated 32 million tons of food waste (U.S. EPA 2011), or 12.7% of total U.S. MSW generation. Florida alone generated 1.8 million tons in 2008 (FDEP 2011), which accounts for 6% of Florida's total MSW generation. Nationally, only 3% of this food waste was recycled, while in Florida only 1% was recycled; the remaining 99% of food waste was predominantly sent to landfills. A substantial amount of food waste is also sent to sewage treatment plants via food waste disposers in homes, restaurants, and grocery stores. Food waste, which has a high organic content, can significantly increase the energy required to aerobically treat the waste at a wastewater treatment plant. The amount of food waste sent to the sewer is not accounted for in Florida, but this food waste is in addition to the 1.8 million tons sent to landfills. There is enormous potential in Florida to capture unrecycled food waste and utilize it for beneficial reuse. Florida currently has a goal to recycle 75% of MSW by 2020 (FDEP 2011). In order to meet this goal, organic waste, including food waste, must be included in Florida's recycling plan. Diverting food waste from landfills has already been made mandatory in some areas. In 1993, Germany banned the landfilling of solid waste with a total organic carbon content of more than 3% (EEA 2009). In 2009, the City of San Francisco passed an ordinance (San Francisco Environment Code, Chapter 19) requiring all households and businesses to collect food waste and organics separately for recycling at composting facilities. The United Kingdom is currently considering banning food waste disposal in landfills.

Disposal of food waste in landfills has many negative consequences. One of the most serious problems is the emission of harmful greenhouse gases from food waste decomposition in the landfill. When food waste is placed in the anaerobic conditions of a landfill, methane is generated as a byproduct of bacterial degradation. Methane, a potent greenhouse gas, has a 20-year global warming potential 74 times that of carbon dioxide (IPCC 2007). Methane from landfills, known as landfill gas, has historically been emitted to the atmosphere via evolution through the uncapped landfill surface. Current practices include capping the landfill with a liner and collecting the landfill gas for flaring or, in some cases, combustion for electrical generation. Despite these practices, landfills are the third largest anthropogenic source of U.S. methane emissions. Of the methane that is generated within landfills, 44% is emitted to the atmosphere (U.S. EPA 2011). Food waste is rapidly degradable and can produce methane prior to the landfill capping while the landfill is still being filled, which results in food waste contributing largely to the methane emissions from landfills. Diverting food waste from landfills would significantly reduce landfill methane emissions.

Another major problem resulting from the disposal of food waste in landfills is the generation of landfill leachate. Landfill leachate contains many harmful pollutants from various wastes in the landfills. One of the most critical pollutants is ammonium (Kjeldsen et al., 2002). Ammonium results from the decomposition of proteinaceous materials. Food waste is therefore a significant source of ammonium in leachate. Leachate management is an enormous burden for landfill operators. Groundwater must be continually monitored and leachate must be treated (either on-site or by transportation to a wastewater treatment plant). Diverting food waste from landfills can significantly reduce the burden of treating ammonium in leachate.

Wasting food, regardless of its disposition, can have inherent negative consequences. Food that is not eaten still requires the same amount of water, energy, labor, fertilizer, pesticide, and land to grow as food that is consumed. Energy is needed to transport, process, and cook the food, which contributes to the embodied energy lost when food is wasted (Cuellar & Webber, 2010). The collection of food waste results in odor and vermin problems, which are particularly problematic in urban areas. Wasting food also carries with it many ethical dilemmas, such as the issue of global hunger and malnourishment. While there will always be food waste, reducing food waste and developing beneficial reuse can greatly reduce the problem of food waste.

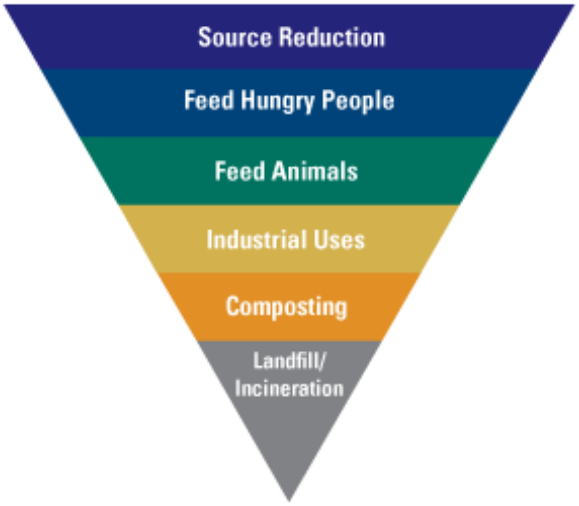


Figure 1-1. Food waste recovery hierarchy (U.S. EPA 2006). Note: Industrial uses include anaerobic digestion.

There are many options for diverting food waste from landfills and creating beneficial reuse, including source reduction, donation to food banks, use as animal feed, and composting. The U.S. EPA has developed a food waste recovery hierarchy outlining various recovery and disposal options for food waste (Figure 1-1). Food waste can be used as a source of sustainable bioenergy and biofertilizer. The process of anaerobic digestion can convert food waste into methane and soluble nutrients through microbial degradation. Anaerobic digestion has typically been utilized in the U.S. for animal manure and municipal sludge. Food waste, however, makes

an excellent feedstock due to its high organic content, and the diversion of food waste for anaerobic digestion reduces the negative environmental, social, and economic consequences of food waste while creating two beneficial end-products (Graunke & Wilkie, 2008).

Anaerobic Digestion

Anaerobic digestion is a microbial process that utilizes anaerobic fermentation and anaerobic respiration to metabolize organic materials into methane and carbon dioxide, which collectively are called biogas. During the process, nutrients within the organic material are released into soluble forms and contained within the digester effluent. The nutrient-rich effluent (known as biofertilizer) can be used as a replacement for fossil-fuel-based synthetic fertilizers. Reusing the nutrients from food waste prevents those nutrients from entering landfills where they become pollutants in leachate and possibly groundwater. Instead, biofertilizers from food waste promote organic and sustainable agriculture by using nutrients captured from waste. Anaerobic digestion of food waste creates a closed-loop, sustainable cycle for the beneficial reuse of food waste (Figure 1-2).

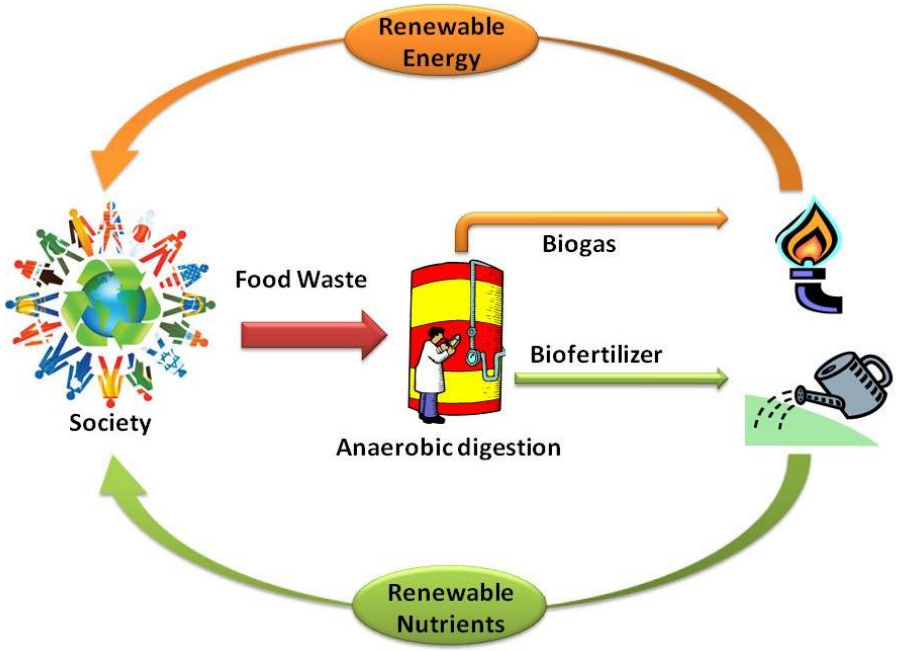


Figure 1-2. Closed-loop cycle created by the anaerobic digestion of food waste.

Anaerobic Digesters and Operating Conditions

Anaerobic digester is a term used to describe a wide variety of reactors that contain the microbial ecology necessary for anaerobic digestion to occur. Some common types of anaerobic digesters include batch reactors, continuously-stirred tank reactors (CSTR), up-flow anaerobic sludge blanket (UASB) reactors, plug-flow reactors, covered lagoons, two-phase digesters, and fixed-film digesters. The performance and operating conditions depend upon the digester type and the feedstock used in the process. Important operational parameters for anaerobic digesters are temperature, hydraulic retention time (HRT), pH, organic loading rate (OLR), methane production rate, and methane yield.

Anaerobic digesters can be generally classified by operating temperature as mesophilic (25-45°C) or thermophilic (45-60°C) digesters. The microbial ecology that exists in a digester is dependent on the temperature at which it is operated. For example, mesophilic digesters contain mesophilic microbial consortia, which are comprised of different organisms than those adapted to thermophilic conditions. In general, thermophilic digesters have faster kinetics and methane production rates than mesophilic digesters due to increased microbial metabolic rates.

Digesters are also classified by HRT. Hydraulic retention time is the amount of time the feedstock is held within a digester for the desired extent of degradation and methane yield. The HRT is typically in units of days and is calculated by dividing the total digester volume by the volume fed per day. Two critical factors that contribute to the HRT of a digester are the feedstock and microbial population. Less degradable feedstocks, such as those with high particulate matter, require more time in a digester due to the time required to solubilize the material. Continuously-stirred tank reactor digesters, for example, generally require longer HRTs due to low microbial populations. High-rate digesters, such as fixed-film reactors, which contain media for attached growth, have low HRTs due to increased microbial populations.

One of the most important parameters controlling digestion is pH. The methanogens that are responsible for methane production in the digester have a specific optimum pH range (6.5-8.2) in which they can thrive. At a pH below this range, the activity of methanogens is sharply reduced (Speece, 2008). This reduces the conversion of organic acids to methane, and the digester becomes acidified or stuck. A drop in pH can occur through overloading the system beyond the maximum OLR. The OLR is the rate at which the feedstock is loaded in the digester, which is based upon the rate at which the microbial consortia can metabolize the feedstock to methane. In anaerobic digestion, bacteria ferment organic matter into organic acids prior to methanogenesis, and the rate of acid production typically exceeds the rate of methane production. By exceeding the maximum OLR of a digester, increased acidogenesis causes accumulation of organic acids, which can decrease the pH and inhibit methanogenesis. This situation is a positive feedstock loop in which methanogenic inhibition further reduces organic acid conversion to methane and cause further acidification.

Acidification is particularly problematic for food waste digestion due to the high organic concentration of food waste. The tendency of food waste to acidify is one reason why food waste digestion does not exist on the commercial scale with food waste as the sole feedstock. Existing facilities co-digest food waste with manure or municipal sludge. Manure and sludge have a higher alkalinity than food waste and act as a pH buffer for the high organic acid concentrations from food waste. While the process may be acceptable in some situations, co-digestion with manure or sludge is not always an option for food waste digestion. In urban areas, where food waste is prevalent, these materials might not always be available, and there is limited space for large co-digestion facilities. The addition of manure or sludge to food waste digesters presents other issues, such as pathogens, antibiotics, and heavy metals in the feedstock and

resulting biofertilizer. Optimizing the microbial kinetics of food waste digestion is imperative for implementing commercial-scale food waste digestion without the use of manure or municipal sludge.

Anaerobic Microbial Consortia

The operating conditions of a digester are dependent on the microbial consortia in the digester. Anaerobic digestion operates through the sequential metabolic processes of mixed microbial consortia and is generally divided into four phases, each with a unique microbial consortium: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1-3).

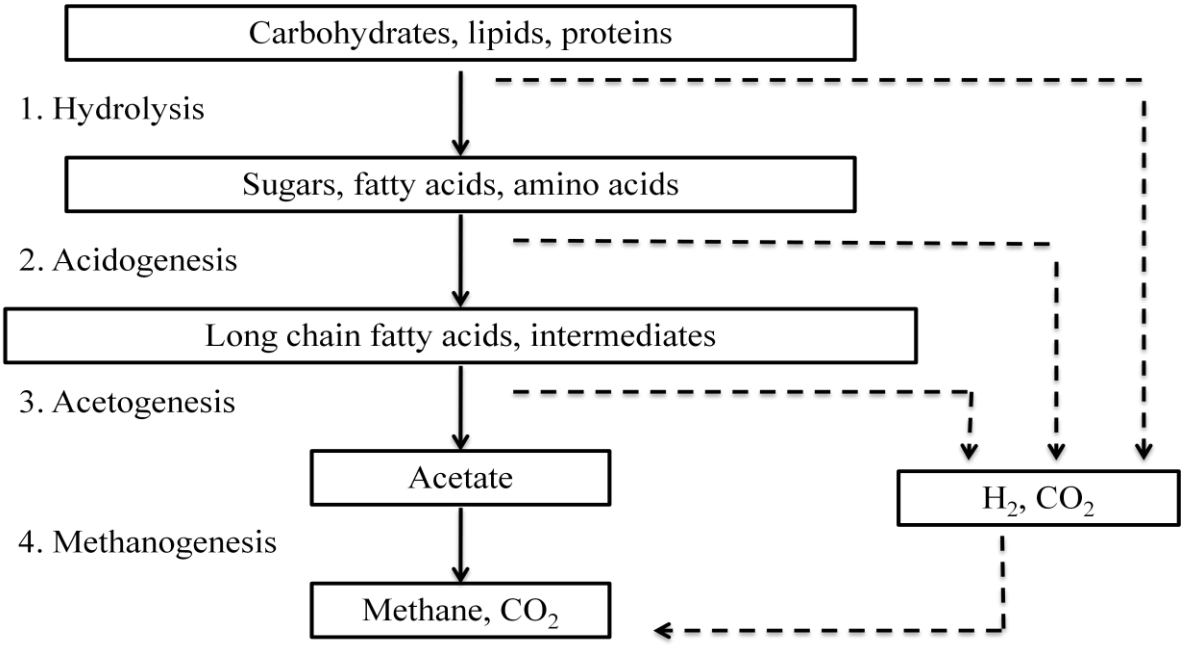


Figure 1-3. Sequential metabolic phases in anaerobic digestion.

Hydrolysis

In order for microbial assimilation to occur, large organic polymers (i.e. carbohydrates, proteins, and lipids) must first be hydrolyzed into their constituent monomeric compounds (i.e. glucose, amino acids, and fatty acids). The hydrolyzed molecules are released into the soluble phase in the digester where they are assimilated by the microbial biomass. In an anaerobic digester, hydrolysis is primarily facilitated by extracellular enzymes (including both cell-free and

cell-bound enzymes) produced by the hydrolytic consortium. Several genera of bacteria have been identified for the production of hydrolytic enzymes in a digester; some of the more common genera include *Clostridium*, *Eubacteria*, and *Pseudomonas* (Lynd et al., 2002; McInerney, 1988). Anaerobic fungi present in the digester can also produce hydrolytic enzymes. In many cases, the hydrolytic bacteria are also acidogenic bacteria and use the hydrolyzed monomers in their own metabolism.

Acidogenesis

Acidogenic bacteria consume the monomeric products of hydrolysis and, through fermentation, produce long-chain and volatile fatty acids (VFAs). Acid fermentation is a complex phase in which a number of different acidogens produce several different organic acids. Other acidogens can consume these acids and ferment them into different organic acids (Kim & Gadd, 2008). Acidogenesis is generally the fastest phase in the digestion process and in an unbalanced digester, increased acidogenesis can cause acidification.

Acetogenesis

There are two primary types of acetogenic bacteria: obligate hydrogen-producing acetogens (OHPA) and homoacetogens (Kim & Gadd, 2008). The OPHA are the more dominant group of acetogens in anaerobic digestion and consume the fatty acid intermediates from acidogenesis to produce acetate through fermentation. The fermentation process also produces CO_2 and H_2. If the concentration of H_2 is too great, OPHA are inhibited from producing acetate. Homoacetogens produce acetate by consuming CO_2 and H_2, which helps to rebalance the metabolism for the OPHA.

Methanogenesis

Methanogenesis occurs through anaerobic respiration by using electrons from acetate and hydrogen produced in acetogenesis to reduce CO_2 to methane (Wilkie, 2008). Specialized

archaebacteria called methanogens comprise the methanogenic consortium in anaerobic digestion. There are two primary types of methanogens: acetoclastic methanogens and hydrogenotrophic methanogens. The acetoclastic methanogens consume acetate to produce methane and carbon dioxide. By consuming the acetate, which is the end product of the acid fermentation process, the acetoclastic methanogens maintain pH balance in the digester. Acetoclastic methanogenesis is responsible for the majority of methanogenesis in most anaerobic digesters (Speece, 2008). Hydrogenotrophic methanogens produce methane from carbon dioxide and hydrogen. They often live in a mutualistic relationship, called syntropy, with the OPHA, by consuming the H_2 from the OPHA. This reduces the concentration of H_2 so the OPHA can metabolize organic acids into acetate, which is then consumed by acetoclastic methanogens, and the pH in the digester is kept in balance. The syntropy between acetogens and methanogens is the driving force for functional anaerobic digestion.

The inevitable result of the sequential metabolism of anaerobic digestion is that the overall rate of anaerobic digestion is dependent upon the slowest of the individual phases. It is widely cited that for the anaerobic digestion of high-particulate feedstocks, such as food waste, hydrolysis is generally considered the rate-limiting step (Eastman & Ferguson, 1981; Izumi et al., 2010; Palmowski & Muller, 2003; Wang et al., 2006). Therefore, to enhance the anaerobic digestion of food waste, the process of hydrolysis is of critical importance.

Hydrolysis and Extracellular Enzymes

Hydrolysis of solid organic material in an anaerobic digester is largely performed through the production of extracellular enzymes by the hydrolytic microbial consortium. These enzymes break bonds within the organic molecules and release the constituent monomers, which are then assimilated by the microorganisms (Figure 1-4). While a large number of different hydrolytic enzymes function in a digester, four important classes of hydrolytic enzymes are cellulases,

amylases, lipases, and proteases. These four classes of enzymes hydrolyze some of the most prevalent components of food waste (Table 1-1). The relative importance of each of these enzyme classes will vary depending on the composition of food waste (Figure 1-5)

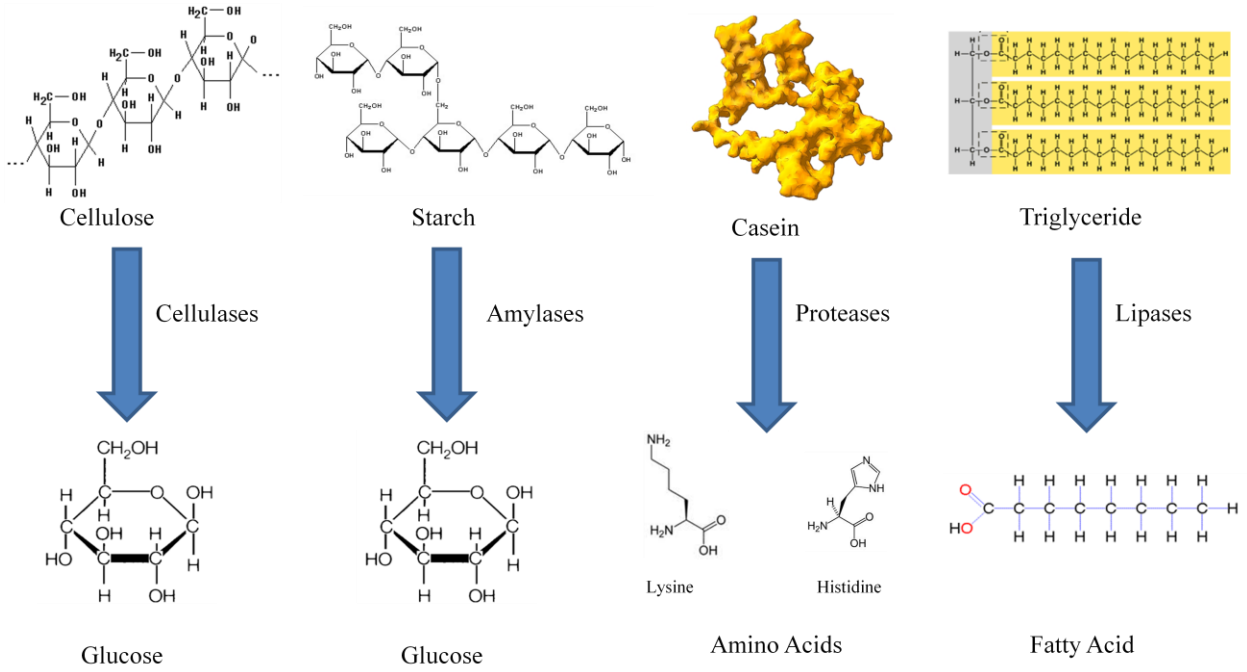
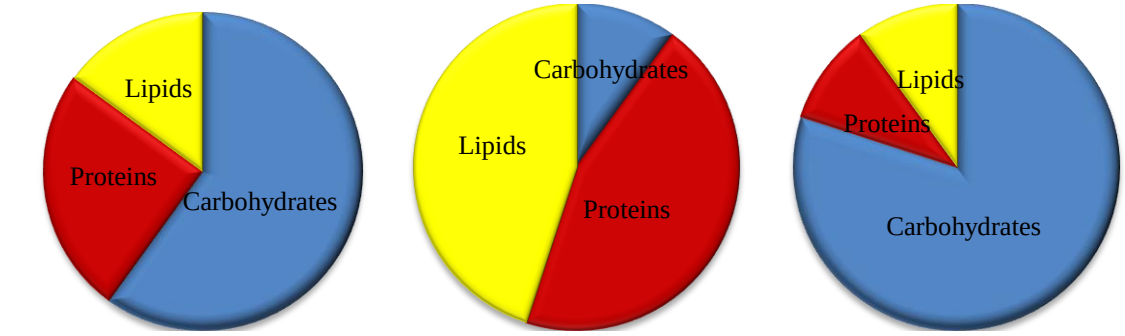


Figure 1-4. The hydrolysis of organic macromolecules into their constituent monomers

Table 1-1. Enzyme classes and target substrates found in food waste

Enzyme class	Target food waste substrates
Cellulases	Broccoli, cabbage, corn cobs, fruit and vegetable trimmings
Amylases	Bread, rice, pasta, potatoes,
Lipases	Oil, dairy products, meat trimmings
Proteases	Meat, legumes, dairy products



A. Restaurant food waste B. Butcher shop food waste C. Bakery food waste

Figure 1-5. The variable composition of food waste. Note: These figures are for illustration only and do not represent measured values.

Cellulases

Depending upon the source, certain food wastes can have a high content of cellulose, particularly kitchen scraps or food processing wastes, which contain the inedible portion of produce, i.e. fruit and vegetable trimmings. Cellulose is a long-chain carbohydrate composed of crystalline glucose polymers. In cellulosic plant material, crystalline cellulose is associated with amorphous regions of hemicelluloses and pectin. Due to its large size and crystalline structure, cellulose tends to degrade slower than more labile materials, such as starch. Cellulases are hydrolytic enzymes produced by cellulolytic bacteria that break the bonds holding the glucose molecules together. In anaerobic conditions, bacteria require close proximity to the cellulose molecule, and cell-bound forms of cellulases are generally produced (Lynd et al., 2002). The bacteria produce a protuberance called a cellulosome that binds to both the bacterial cell wall and the cellulose surface. The cellulosome is a scaffolding protein structure that holds the active sites of the cellulases. Because the cellulosome must attach to the substrate surface, increasing the substrate surface area can increase the opportunities for these attachments to occur.

Amylases

Starch is one of the most prevalent macromolecules found in food waste. Starch is a branched polymer of glucose monomers. Starch has two subunits, amylose, a straight chain molecule and amylopectin, side chain branches. Unlike cellulose, which has a crystalline structure, the branched structure of starch allows for faster hydrolysis. The hydrolysis of starch is facilitated by amylases, which break the bonds between the glucose monomers. Because of the abundance of starch in food waste, amylases are critical for the anaerobic digestion of food waste. Wang et al. (2009) found that high lactic acid levels produced during acid fermentation inhibit amylases, specifically glucoamylases. The enzymes can become denatured through

prolonged lactic acid inhibition. Maintaining the organic acid balance, particularly lactic acid, is important during food waste digestion for proper starch hydrolysis to occur.

Lipases

Lipids are important macromolecules in anaerobic digestion because of their high methane production potential compared with carbohydrates or proteins (Wilkie, 2008). Lipids, however, can be problematic in digesters due to clogging or flotation and interference with the degradation of other substrates (Cirne et al., 2007). Therefore, the hydrolysis and digestion of lipids are important for the optimization of food waste anaerobic digestion. Lipases are extracellular lipolytic enzymes that break the lipid molecule into free fatty acids and glycerol. Lipases function at the lipid-water surface interface by adhering to the lipid molecules (Sharma et al., 2001) in a phenomenon termed interfacial activation. Lipases contain a surface loop structure that acts as a lid to cover its hydrophobic active site. When the lipase binds to the lipid surface, the lid opens and exposes the lipase active site to the lipid. The hydrophobicity of the active site helps to bind the lipase to the lipid surface. Because lipases need direct contact with the lipid molecule, increasing the surface area of food waste increases the opportunities for lipase/lipid interactions.

Proteases

Proteins are important in an anaerobic digester because they have higher methane potential than carbohydrates (Wilkie, 2008), and, because proteins are nitrogenous compounds, their presence in the feedstock increases the fertilizer value of the effluent through the release of ammoniacal nitrogen. Proteases are hydrolytic enzymes that break the peptide bonds between the amino acids of a protein. Protein molecules have a wide variety of structural morphologies, which can facilitate or impede access to the peptide bonds in the protein. Collagen found in meat, for example, has a tightly bound, triple-helix tertiary structure, which greatly reduces the

degradability of the protein. Disrupting the tissue and exposing increased surface area of more recalcitrant proteins, like collagen, can increase access of the proteases to the peptide bonds and facilitate protein hydrolysis.

Factors Influencing Food Waste Hydrolysis

While the presence of hydrolytic bacteria and enzymes is necessary for hydrolysis to occur during anaerobic digestion, several physiochemical factors influence the performance of enzymatic hydrolysis. Three critical factors are pH inhibition, product inhibition, and temperature. As with the microorganisms in a digester, the extracellular enzymes they produce also have an optimum pH range, and these ranges can vary depending on the enzyme. In the sequential metabolism of the anaerobic consortia, the products of hydrolysis are fermented into organic acids. If the rate of acidogenesis exceeds the rate of methanogenesis, organic acids may accumulate and cause a drop in pH, which results in enzyme inhibition and denaturing. In addition, enzymes can be inhibited through product inhibition from accumulation of the hydrolytic and acidogenic products, which causes the product/substrate equilibrium to favor unhydrolyzed substrate. He et al. (2006) studied the effects of pH and acetate inhibition on potato starch. They concluded that carbohydrate hydrolysis was inhibited by low pH, while protein hydrolysis was only inhibited by a low pH in the presence of acetate. Veeken et al. (2000) performed a similar study examining the impact of pH and VFA concentration on the hydrolysis of the organic fraction of municipal solid waste and found the rate of hydrolysis was pH dependent.

Temperature also has a significant impact on the activity of hydrolytic enzymes, which have an optimum temperature range. High temperatures can denature enzymes while low temperatures can halt enzyme activity. Veeken and Hamelers (1999) examined the hydrolytic rate of the organic fraction of municipal solid waste at 20°C and 40°C. A first-order rate

constant of 0.03-0.15/day was found at 20°C, while a rate constant of 0.24-0.47/ day was found at 40°C. The solubilization of food waste was found to be greater at 35°C and 45°C than at 25°C or at thermophilic temperatures (55°C and 65°C) (Komemoto et al., 2009). High temperature can denature enzymes, which may explain why a higher solubilization rate was found at 35°C and 45°C. These studies suggest that mesophilic temperatures are ideal for the solubilization of food waste.

Pretreatment of Food Waste

An important common feature of hydrolytic enzymes is that they require contact with the exposed surface area of the organic substrate. Therefore, one limiting factor for the rate of hydrolysis is the surface area available for enzymatic interaction. Because hydrolysis is considered the rate-limiting step in the anaerobic digestion of high-particulate feedstocks, increasing the substrate surface area and availability to hydrolytic enzymes can enhance the overall kinetics of anaerobic digestion. In order to increase the substrate surface area, numerous pretreatment techniques have been studied. While there is exhaustive literature studying the pretreatment of waste activated sludge or the organic fraction of municipal solid waste, literature addressing the pretreatment of food waste for anaerobic digestion is comparatively sparse.

An important measure when studying hydrolysis is the fractionation of the chemical oxygen demand (COD) of a substrate. Chemical oxygen demand is a surrogate measurement for organic matter content and is used to determine the stoichiometric methane potential of a substrate (1 g COD stoichiometrically produces 0.35 L methane at STP). The total COD (TCOD) of a substrate can be fractionated between soluble COD (SCOD or CODsf as the soluble fraction of TCOD) and particulate COD (PCOD). The endogenous CODsf of a substrate is the soluble material contained within cells and tissues, for example, glucose. Particulate COD can be segregated into labile particulate matter and resistant particulate matter (Figure 1-6).

Examples of labile particulate matter include starches and oligosaccharides, and examples of resistant particulate materials include cellulose, proteins, and triglycerides. As PCOD solubilizes by enzymatic hydrolysis, the CODsf of the substrate increases. Organic materials also contain recalcitrant material that has very low degradability. Recalcitrant organic matter can be either soluble (e.g. tannins) or particulate (e.g. lignin).

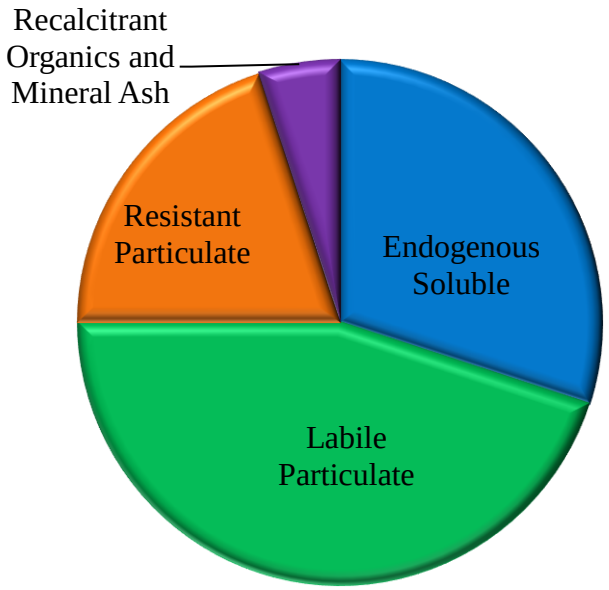


Figure 1-6. Food waste composition by degradability. Note: Figure is for illustration only and not based on measured values.

Because the anaerobic consortia can only assimilate soluble material, determining the rate at which the endogenous SCOD is released and the rate at which PCOD is hydrolyzed into SCOD is critical in determining the anaerobic digestion kinetics of the substrate. To illustrate the effects of CODsf on methane kinetics, Prashanth et al. (2006) conducted a study digesting a synthetic substrate. The substrate consisted of cellulose (PCOD) and sucrose and peptone (SCOD). These were loaded at different initial CODsf (100, 75, 50, 25, and 0%) and were loaded at varying feed/inoculums ratios. After 60 days of digestion, the cumulative methane (at

STP) produced by 0% CODsf (pure cellulose) ranged from 92-248 mL/g COD, depending on inoculum loading rate. This is significantly below the stoichiometric maximum of 350 mL/g COD. When CODsf was 100%, 60 day cumulative methane production was 264-350 mL/g COD. A CODsf of 75% resulted in 87%-91% of the methane produced by 100% CODsf. Below CODsf of 75%, methane production dropped significantly. The authors concluded that at a CODsf less than 75%, hydrolysis is considered to be rate-limiting. Therefore, by increasing the CODsf of food waste, the kinetics of anaerobic digestion are more favorable. This can be accomplished by two means, increasing the release of endogenous SCOD from the material and increasing the substrates availability to hydrolytic enzymes. In order to increase the CODsf, a variety of pretreatment methods, including thermal, freezing/thawing, enzymatic and mechanical, have been reported in the literature.

Thermal and Freezing/Thawing Pretreatment

One method of increasing food waste solubilization is through thermal pretreatment. Heating food waste disrupts tissue and ruptures cells, which releases SCOD and increases surface area for enzymatic hydrolysis. Wang et al. (2006) found that thermal pretreatment increased solubilization and biomethanation of food waste in a two-phase digester (a two-phase digester separates the acidogenic phase from the methanogenic phase in two reactors). Heating shredded food waste to 70°C for 2 h or 150°C for 1 h increased the initial CODsf, through endogenous SCOD release, from 17.2% for control (shredded food waste) to 20.3% and 31.7% respectively, for 70°C and 150°C pretreatments. The study also found increased methane production with thermal pretreatment; the digestion time to produce the same volume of methane decreased 33.3% with 70°C pretreatment and 50% with 150°C pretreatment compared with control.

Liu et al. (2008) studied the effects of heating and freezing/thawing food waste prior to digestion in a two-phased anaerobic digester. When food waste freezes, ice crystals form that rupture cells and tissues. When the food waste thaws, endogenous SCOD is released. They found that thermally pretreated, shredded food waste (150°C for 1 h) and frozen/thawed, shredded food waste (24 h at -20°C then 12 h at 25°C) both showed (via SEM micrographs of the food waste) increased porosity and looser cell structure, which increased surface area and availability for hydrolytic enzymes. Both pretreatments also showed an increased release of endogenous SCOD. Freezing/thawing increased the initial CODsf from 14.9% (control, shredded food waste) to 25.4%. Thermal pretreatment increased initial CODsf from 7.8% (control, shredded food waste) to 16.9%. The SCOD was measured daily on the leachate from the acidogenic reactor. Cumulative SCOD production over the first 6 d was 13% and 39% greater than control for thermally pretreated food waste and frozen/thawed food waste, respectively. This indicated that pretreatment also enhanced the enzymatic hydrolysis of food waste. The increased solubilization kinetics showed corresponding increases in biomethanation kinetics. Total methane produced over 12 d increased by 29% over control with thermal pretreatment and 11% with freezing/thawing. To produce the same volume of methane, thermal pretreatment decreased operational time by 48%, while freezing/thawing decreased operational time by 42%.

Both of these studies indicated that thermal pretreatment and freezing/thawing can significantly increase the release of endogenous SCOD and substrate availability for hydrolytic enzymes, resulting in increased kinetics for solubilization and methane generation. However, thermal pretreatment and freezing/thawing are both energy intensive methods of pretreatment. Liu et al. (2008) performed an energy balance between the energy required for pretreatment and

the energy gained through increased methane kinetics, which showed the energy used offset the energy gained. Therefore, the net benefit of thermal and freezing/thawing pretreatment is nullified. Another problem with these pretreatment methods is that the endogenous enzymes and microorganisms in the food waste, which contribute to hydrolytic enzymes and the microbial consortia of the digester, can be denatured and killed during the pretreatment.

Enzymatic Pretreatment

Enzymatic pretreatment has also been studied to increase solubilization of food waste. Because solubilization in anaerobic digestion is facilitated by hydrolytic enzymes, the addition of these enzymes as a pretreatment step can enhance food waste solubilization. Kim et al. (2005) used an enzyme cocktail of commercial carbohydrases, lipases, and proteases for enzymatic pretreatment of food waste. They found that blended food waste pretreated with 0.4% (v/v) enzyme cocktail resulted in a maximum CODsf of 68.5%, while an untreated control (blended fresh food waste) had a CODsf maximum of 37.5%. The enzyme treatment reached maximum solubilization within 4 d, with 61% of SCOD produced within the first 12 h; the control, however, plateaued within the first few hours. Enzymatic pretreatment may increase food waste solubilization; however, the cost of enzymes may be prohibitive for commercial-scale implementation of food waste anaerobic digestion. Because food waste composition varies, a unique enzyme cocktail may be required for each type of food waste.

If necessary, enzyme pretreatment of food waste is most suited for specialized or unique food waste as a boutique pretreatment method. One example where enzymatic pretreatment may be useful is for high-lipid food waste streams. Because of their hydrophobic nature, lipids can bind to other substrates and microbial biomass in the digester. This action inhibits digestion of the feedstock, reduces methane production, and can cause clogging problems, as was demonstrated by Neves et al. (2008). The study examined a standard food waste with an excess

of lipids, cellulose, proteins, or carbohydrates added in different formulations. The food waste with an excess of lipids showed a much slower biomethanation rate. To reach 50% degradation with excess lipids, 14.8 d were required, while all other food waste formulations required 3.0-5.9 days. To reach 85% degradation with excess lipids, 57 d were required, while other food waste formulations required 23-32 d. Because of the slow kinetics of lipid hydrolysis and digestion, as well as their potential to inhibit digestion of other substrates, the authors recommended employing special measures with high-lipid food wastes. One option would be the application of lipases. In lab-scale experiments, the application of pancreatic lipases to slaughterhouse waste has a positive effect on reducing particle size of fat molecules prior to anaerobic digestion (Masse et al., 2001). Mendes et al. (2006) applied pancreatic lipases to dairy processing waste with a high lipid concentration, and found positive results in hydrolytic and methanogenic kinetics. Within 12 h of lipase treatment, 39.5% of lipids were hydrolyzed. The 15 d cumulative methane production of lipase-treated waste was over twice that of the treatment control. For the anaerobic digestion of high-lipid food waste, lipase addition may be a beneficial pretreatment method. However, for generic food waste (i.e. restaurant, grocery store, and kitchen waste), enzymatic pretreatment may be a costly and unnecessary step when appropriate mechanical pretreatment and particle size reduction is applied.

Mechanical Pretreatment

Mechanical pretreatment functions through particle size reduction and tissue and cell maceration by various mechanical devices. Because hydrolysis is mediated by extracellular enzymes and their interaction with substrate surface area, increasing the surface area through mechanical pretreatment can increase food waste solubilization. Studies have shown the importance of particle size on the kinetics of anaerobic digestion of food waste. Sanders et al. (2000) measured the solubilization of potato starch particles in batch experiments. They found

that the rate of solubilization of starch particles is directly related to the surface area. Palmowski and Müller (2003) tested the effect of communition on the anaerobic digestion of various organic materials, including apple and rice, in 1 L batch assays. They found that, through communition, there was an increase in cumulative biogas production from the anaerobic digestion of apple and rice and a nearly 40% and 30%, respective decrease in digestion time over control. Analysis of the specific surface area, through nitrogen adsorption, of rice showed that there was a positive correlation between substrate surface area and endogenous SCOD released. Because particle size and surface area are critically important in the anaerobic digestion of potato starch, apples and rice, the use of mechanical pretreatment for food waste should show similar results.

Izumi et al. (2010) examined the effects of mechanical pretreatment on a standard food waste. The food waste was pretreated using a household food disposer (control) and then further pretreated using a ball mill at different numbers of revolutions. The mean particle size after disposer treatment was 0.888 mm, while additional ball milling for 300 revolutions resulted in mean particle size of 0.843 mm. The particle size decreased with increased ball milling, to reach a minimum of 0.393 mm with 40,000 revolutions. Ball milling also increased the release of endogenous SCOD of the food waste over disposer pretreatment alone. Food waste pretreated with the disposer alone had an initial solubilization of 28.1%; additional pretreatment with the ball mill at 300 revolutions increased solubilization to 39.8%. However, the decrease in particle size with increased ball milling revolutions did not seem to increase the release of endogenous SCOD; with 40,000 revolutions, the initial solubilization was 40.3%. This suggested that the entirety of the endogenous SCOD was released with minimal ball milling and was not correlated with decreased particle size by further pretreatment.

The authors also explored the effect of mechanical pretreatment on methane production. The pretreated food waste was digested for 16 days under mesophilic conditions at a loading rate of 10 g COD/L in lab-scale batch anaerobic digesters. Biogas production and pH were measured over the course of the digestion period. The decrease in particle size with the additional pretreatment of ball milling had a positive effect on methane production. Cumulative methane production for disposer treatment alone was 251 mL/g COD (at STP), while disposer and ball milling for 1000 revolutions had a cumulative methane production of 322 mL/g COD (at STP). With increased ball milling above 1000 revolutions, however, methane production decreased. At 40,000 revolutions cumulative methane was only 254 mL/g COD (at STP). The 40,000 revolutions treatment also showed the most significant initial drop in pH and the slowest recovery rate. The authors measured VFA concentration and found that the 40,000 revolutions treatment had a total VFA (acetic, propionic, n-butyric, i-butyric, n-valeric, i-valeric) concentration of 5,600 mg/L. This indicates that with excessive pretreatment, acidification through VFA accumulation can occur as the rate of hydrolysis and acidogenesis is greater than the methanogenic rate. This study suggests that moderate mechanical pretreatment can enhance the anaerobic digestion of food waste by overcoming the rate-limitation of hydrolysis; however, excessive pretreatment can increase the hydrolytic and acidogenic rates to a point where they exceed the methanogenic rate and inhibit methanogenesis. The practical applications of this study are questionable because ball milling, while potentially increasing digestion kinetics, is not feasible for pretreatment in commercial-scale food waste digestion. Therefore, there is a need to study how practical pretreatment methods can increase the solubilization and biomethanation kinetics of food waste.

Thesis Rationale

The existing literature suggests that for the anaerobic digestion of food waste, hydrolysis is the rate-limiting step. Food waste has a high particulate fraction that must be hydrolyzed and solubilized prior to microbial fermentation and methanogenesis. Because hydrolysis of particulate matter is facilitated by extracellular hydrolytic enzymes, increasing the availability of the food waste to these enzymes can enhance solubilization and consequently methanogenesis. Additionally, disrupting tissue and rupturing cells can increase the immediate release of endogenous soluble material. Literature suggests that various means of food waste pretreatment can have a positive effect on solubilization and methane generation. One of the major weaknesses in the methods studied is that the pretreatment methods are often not practical for commercial-scale implementation. For example, thermal pretreatment requires substantial energy consumption and ball milling of food waste is not suitable for digestion at other than lab-scale. Another issue with the previous studies is that none examined intact food waste as a control. In all the studies, the control food waste was itself pretreated (i.e. shredded or ground) prior to the pretreatment methods examined. Therefore, the true impact of the pretreatment method tested was obscured because the food waste was in fact pretreated prior to the pretreatment experiment.

Considering these issues, this current effort sets forth to examine the enhancement of food waste solubilization and biomethanation through low-tech, practical mechanical pretreatment methods. The pretreatment methods were selected with implementation in mind so that any resulting enhancement of anaerobic digestion could be expected on-site at a restaurant, school or grocery store. In many cases, these locations already have pretreatment apparatuses on-site (i.e. food disposer or meat grinder) so pretreatment for a food waste digester could fit within existing infrastructure. Additionally, the current work compares the effects of mechanical pretreatment

against intact, fresh food waste. By comparing pretreated food waste to intact food waste, the true impact of pretreatment on solubilization and biomethanation can be measured.

Hypothesis

Mechanical pretreatment of food waste will disrupt tissues and rupture cells, which will enhance food waste solubilization through increased release of endogenous soluble material and greater availability to hydrolytic enzymes. Enhanced solubilization kinetics can increase biomethanation through increased generation of soluble materials for metabolism by the anaerobic consortia.

Objectives

The goal of this study is to examine to the effects of mechanical pretreatment on the anaerobic digestion of food waste. There are two primary objectives that will be addressed: 1) assess the effects of pretreatment on solubilization kinetics of food waste and 2) assess the effects of pretreatment on biomethanation kinetics of food waste.

CHAPTER 2
MATERIALS AND METHODS

Standard Food Waste

Empirical evidence has indicated that food waste is a highly variable substrate. Food waste audits were conducted at local schools and restaurants over a two week period. The daily-generated food waste was analyzed for total solids (TS), volatile solids (VS), total chemical oxygen demand (TCOD), total nitrogen (TN), and total phosphorus (TP) (Table 2-1) in order to develop a parameter range of food waste collected in the community. Due to the variability of food waste, it was deemed necessary to develop a standard food waste as a substrate for repeatable experimentation. The standard food waste was developed to be within the range of the physiochemical properties of food waste collected during the waste audits. The standard food waste also contained components representing various constituent macromolecules of food waste (i.e. carbohydrates, lipids, and fats). For each experiment, the standard food waste was formulated immediately prior to the experiment in order to fully capture the degradability of fresh food waste. Precautions were taken (e.g. produce was washed, gloves were worn when handling) to reduce microbial contamination that could impact the integrity of the food waste.

Table 2-1. Range of physiochemical properties of food waste

Property	Schools (n=30)	Restaurants (n=50)
Total solids (TS) (%)	25.45 - 42.57	10.64 - 41.55
Volatile solids (% TS)	84.71 - 95.09	85.63 - 97.73
Total chemical oxygen demand (g/kg wet weight (ww))	342.04 - 511.96	127.67 - 565.94
Total nitrogen (% TS)	2.03 - 4.44	2.17 - 3.95
Total phosphorus (% TS)	0.32 - 0.75	0.23 - 0.44

Composition

The standard food waste used in the experiments consisted of six components: apple, potato, bread, broccoli, beans, and cheese (Table 2-2). These ingredients were proportioned

empirically to simulate actual food waste. Each ingredient was selected to represent a range of different types of macromolecules found in food waste.

Table 2-2. Composition of standard food waste

Components	Percent composition (ww)	Percent composition (TCOD)	Macromolecule represented
Apple, Red Delicious	24	11.9	Carbohydrate (sugar, pectin)
Potato, Russet	24	13.7	Carbohydrate (starch)
Beans, red kidney (canned, drained and rinsed)	20	21.2	Protein
Broccoli (florets)	12	4.28	Carbohydrate (cellulose)
Bread, white hamburger bun	12	29.7	Carbohydrate (starch)
Cheese, sharp Cheddar	8	19.2	Protein, lipid

Analysis

Physiochemical properties were measured on the standard food waste to confirm that it was analogous to the food waste collected in the waste audits. Total solids, VS, TCOD, SCOD, conductivity, alkalinity, TN, and TP were measured on the standard food waste (Table 2-3).

Table 2-3. Physiochemical properties of standard food waste

Property	Mean ± Standard Deviation
Total Solids (TS) (%)	29.94 ± 0.20
Volatile solids (% TS)	95.41 ± 0.001
Total chemical oxygen demand (g/kg ww)	348.89 ± 22.87
Soluble chemical oxygen demand (g/kg ww)	87.9 ± 2.7
Conductivity (mS/cm)	4.37 ± 0.16
Alkalinity (mg $CaCO_3$ eq./kg ww)	1344.8 ± 3.67
Total nitrogen (% TS)	3.08 ± 0.12
Total phosphorus (% TS)	0.32 ± 0.0008

Note: All parameters were measured in triplicate

Food Waste Microscopy

Intact and pretreated food waste was examined microscopically to observe the structural changes in the cells and tissues through pretreatment. Four components of the standard food waste (apple, bean, broccoli, and potato) were individually observed. For intact food waste, small cross-sections were delicately cut using a scalpel to preserve cell integrity. For pretreated

food waste, each component was ground using a manual meat grinder with 0.5 cm plate openings (Grizzly H7778 No 32, Grizzly Industrial, Inc., Bellingham, WA). A small sample was placed on a microscope slide with glass coverslip. Photos were taken under darkfield illumination on a Nikon Labophot compound microscope (Nikon Corporation, Tokyo, Japan) using a Spot Insight color mosaic digital camera (Diagnostic Instruments Inc., Sterling Heights, MI).

Solubilization Assay

To study the impact of pretreatment on food waste solubilization and hydrolysis, three solubilization assays were conducted: an endogenous solubilization assay, commercial enzyme assay, and microbial inoculum assay. The full treatment regime is shown in Table 2-4. Each treatment was replicated in triplicate. A phosphate buffer (0.5 M at pH 6.5) was used to prevent acidification, which can inactivate and denature hydrolytic enzymes. Food waste was combined with the other components of the assays (i.e. buffer, DI water, enzymes, and/or inoculum), and placed in a 1 L glass beaker. Beakers were covered with plastic film (Glad ® Cling Wrap) held with a rubber band to reduce evaporation and potential contamination. Assays were maintained at 35°C in a static water bath (Versa-Bath S Model 236, Thermo Fisher Scientific, Waltham, MA). Experimental blanks of commercial enzyme and microbial inoculum were included in the assay to account for non-food waste SCOD.

Table 2-4. Treatment regimes for solubilization assays

	Endogenous solubilization	Commercial enzyme	Microbial inoculum
Food waste	10 g (ww)	10 g (ww)	10 g (ww)
Phosphate buffer	250 mL	250 mL	N/A
Commercial enzyme	N/A	1 g	N/A
Microbial inoculum	N/A	N/A	990 mL
Total volume	1 L (640 mL DI water)	1 L (640 mL DI water)	1 L
Loading Rate	3.5 g COD/L	3.5 g COD/L	3.5 g COD/L
Treatments	Grinder (0.5 cm plate) Grinder (1.0 cm plate) Disposer Intact	Grinder (0.5 cm plate) Grinder (1.0 cm plate) Disposer Intact	Grinder (0.5 cm plate) Intact

Mechanical Pretreatment

Three mechanical pretreatment methods were studied in the endogenous solubilization and commercial enzyme solubilization assays (Table 2-5): an in-sink food disposer (Badger 9 model Insinkerator, Racine, WI), and a manual meat grinder with two different plates (0.5 cm and 1.0 cm plate openings) (Grizzly H7778 No 32, Grizzly Industrial, Inc., Bellingham, WA). Based on results from the first two assays, the grinder with the 0.5 cm plate was selected as the representative pretreatment method for the microbial inoculum assay. To ensure representativeness, 500 g of the standard food waste was formulated and ground separately for each pretreatment method. Triplicate 10 g (ww) mixed, representative samples from each 500 g batch of pretreated food waste were used in the assay. Pretreatment occurred just prior to the assay in order to measure the immediate solubilization kinetics of the food waste. The nominal time required for pretreatment was 0.1 h. For intact food waste, a kitchen knife was used to cut a single, cubic piece of each component needed to formulate 10 g (ww) of the standard food waste, for example, a 2.4 g cube of potato and 0.8 g cube of cheese. Any variation in TCOD was measured in triplicate on each formulation of the standard food waste and accounted for when calculating percent solubilization (CODsf).

Commercial Enzyme

To measure enzymatic hydrolysis in the solubilization assay, a commercial digestive enzyme powder (Digest®, Enzymedica Inc., Port Charlotte, FL) was employed. The formula included a variety of hydrolytic enzymes including cellulases, amylases, proteases, and lipases. The entire enzyme formulation is shown in Table 2-5. According to a customer representative from Enzymedica Inc., the enzyme sources were *Aspergillus niger* and *Aspergillus orzaye*. Prior to use in the assay, the requisite amount of enzyme capsules were emptied and enzyme powder within was completely mixed to ensure representativeness.

Table 2-5. Commercial enzyme cocktail formulation

Enzyme	Concentration (per 0.25 g)	Unit definition
Amylase	12,000 DU	Dextrinizing units
Protease	42,000 HUT	Hemoglobin units on a tyrosine base
Lipase	500 FCC-LU	Food Chemical Codex lipase units
Cellulase	200 CU	Cellulase units
Lactase	850 ALU	Acid lactase units
Alpha Galactosidase	75 GALU	Alpha-galactosidase units
Maltase	200 DP	Diastic power units
Invertase	175 INVU	Invertase units
Pectinase	50 Endo-PGU	Endo-polygalacturonidase units

Microbial Inoculum

The microbial inoculum used in the solubilization assay was formulated by mixing 10 g (ww)/L of standard food waste (ground with 0.5 cm plate meat grinder), 100 mL/L of flushed dairy manure (UF Dairy Unit, Hague, FL), 250 mL/L of phosphate buffer (0.5 M at 6.5 pH), and 640 mL/L of DI water . The inoculum was placed in crimped-capped serum bottles (Wheaton, Millville, NJ) and incubated at 35°C in a water bath (Versa-Bath S Model 236, Thermo Fisher Scientific, Waltham, MA) for 30 days to allow microorganisms to acclimate to the food waste and to produce extracellular hydrolytic enzymes. Physiochemical properties of the inoculum are shown in Table 2-6. One day prior to assay, serum bottles were opened and inoculum was screened using a 20-mesh wire sieve (850 µm opening) (Newark Wire Cloth Co., Newark, NJ) to remove large particulates. Screened inoculum was placed into two 4 L glass beakers, covered with plastic film (Glad ® Cling Wrap), and held at 35°C overnight prior to the assay. Inoculum was continuously mixed using a magnetic stir bar while adding to food waste in the assay.

Table 2-6. Physiochemical properties of microbial inoculum used in solubilization assay

Property	Microbial Inoculum
Source	10 % flushed dairy manure with food waste
TCOD (mg/L)	2932 ± 77.5
SCOD (mg/L)	1685 ± 41.9
pH	6.46

Sampling Technique

At 0, 1, 2, 4, 6, 8, 12, and 24 h, SCOD and pH were measured. Each replicate was removed from the water bath, one at a time, and the plastic film cover was removed. The replicate was thoroughly mixed using a magnetic stir bar, and a 20 mL aliquot was sampled for SCOD analysis. The aliquot was immediately placed into a refrigerated water bath (Refrigerated Bath Model 90, Thermo Fisher Scientific, Waltham, MA) at 4°C to quench enzyme activity. Following aliquot sampling, pH was measured on the replicate. The plastic film cover was replaced and replicate was returned to the water bath.

Statistical Analysis

Solubilization and hydrolysis were fit to first-order kinetic models using non-linear fitting.

$$\mathrm{CODsf} = \mathrm{CODsf_f} * (1 - \mathrm{e}^{(-\mathrm{k}*\mathrm{t})})$$

Where:

- CODsf= COD soluble fraction at time t (%)
- $\mathrm{CODsf_f}$=ultimate CODsf (%)
- k=kinetic rate constant ($\mathrm{h^{-1}}$)

The solver application in Microsoft Excel 2007 was utilized to solve for $\mathrm{CODsf_f}$ and k by minimizing the residual sum of squares of triplicate data points. Standard error of each parameter was calculated using the square root of the variance. A Student's t-test (α=0.05) was used to determine significant differences between mean values of parameters.

Biochemical Methane Potential Assay

Biochemical methane potential (BMP) assays were conducted to determine the effect of pretreatment on the methane production kinetics of food waste. The BMP assay used in this study is a modification of the methods developed in Owen et al.(1979). Two BMP assays were conducted on pretreated and intact food waste: a moderate-loading-rate assay and a reduced-loading-rate assay. Table 2-7 shows the full assay regime for each BMP assay. The moderate-

loading-rate and reduced-loading-rate BMP assays were loaded with the standard food waste at nominal organic loading rates of 3.5 g COD/L and 2 g COD/L, respectively. The assays were conducted using glass serum bottles with a nominal volume of 534 mL (Wheaton, Millville, NJ). Food waste was either intact or pretreated using a meat grinder with 0.5 cm plate openings per the methodology used for the solubilization assays. For the moderate-loading-rate assay, bottles were inoculated with flushed dairy manure. For the reduced-loading-rate assay, the digestate from the moderate-loading-rate assay was conserved and reused as an inoculum. While mixing, 400 mL of the inoculum was poured into each bottle. Positive controls were run using glucose and cellulose at a stoichiometric loading rate of 2.13 g COD/L and 2 g COD/L for the moderate-loading-rate and reduced-loading-rate assays, respectively, with 400 mL of inoculum. An experimental blank was included in each assay to account for methane production from the inoculum. All treatments, controls, and blanks were conducted in triplicate. Once all bottles were loaded, they were sealed with a rubber septum stopper and an aluminum crimp cap (Wheaton, Millville, NJ). Bottles were inverted to prevent potential gas leakage and placed into a 35°C incubator. Bottles were manually shaken once daily.

Table 2-7. Biochemical methane potential assay regime

	Moderate-loading-rate BMP	Reduced-loading-rate BMP
Pretreated loading rate (g COD/L, measured)	3.55	2.05
Intact loading rate (g COD/L, measured)	3.48	2.18
Glucose and cellulose loading rate (g COD/L)	2.13	2.00
Inoculum added to each bottle (mL)	400	400

Simulated BMP Assay for pH Measurements

Following the moderate-loading-rate BMP assay, a simulated BMP assay was conducted in which subsamples were taken for 20 days from each bottle for measurement of pH. Methane production was not measured in the simulated assay. The assay was conducted in triplicate with

pretreated and intact food waste loaded at three different loading rates: 2, 4, and 8 g COD/L. Bottles were manually mixed during sampling and a 10 mL aliquot was taken by piercing the septum with a stainless steel needle (19 gauge) and 12 mL syringe. Aliquot pH was measured immediately with limited agitation or mixing to reduce CO_2 evolution. Bottles were manually degassed to prevent acidification through CO_2 accumulation. The inoculum in the simulated BMP assay was flushed dairy manure.

Methanogenic Inoculum

The inoculum used in the moderate-loading-rate assay was flushed dairy manure obtained from the University of Florida Dairy Unit (Hague, FL). In the reduced-loading-rate assay, the digestate from the moderate-loading-rate assay was conserved and eused as an inoculum. Prior to use in each assay, inoculum was thoroughly mixed and screened using a 20-mesh wire sieve (850 µm opening) (Newark Wire Cloth Co., Newark, NJ) to remove large particulates. A 400 mL mixed, representative aliquot of screened inoculum was used in each replicate of the assay. Total COD, SCOD, and pH of each BMP inoculum are presented in Table 2-8.

Table 2-8. Physiochemical properties of inocula for the biochemical methane potential assays

Property	Moderate-loading-rate BMP	Reduced-loading-rate BMP
Source	100% Flushed dairy manure	Digestate of moderate-loading-rate BMP
TCOD (mg/L)	4108 ± 80.8	2582 ± 64.2
SCOD (mg/L)	620 ± 9.9	487 ± 6.3
pH	7.54	7.82

Methane Production Measurement

Methane production was measured volumetrically through hydraulic displacement using a modification to methods described in Wilkie et al. (2004). Bottles were removed from the incubator and measured individually. The apparatus for methane measurements (Figure 2-1) consisted of a short, clamped tube (B) with a needle on either end (C) connected between the BMP serum bottle (A) and an inverted 250 mL serum bottle filled with 5M KOH (D). The tube

was opened, allowing the biogas to flow from the BMP bottle and bubble through the 5M KOH. By passing through 5M KOH, carbon dioxide was stripped out of the biogas into solution leaving only methane in the headspace of the KOH bottle. Alizarin was added to the KOH solution at a rate of 1g/L as a pH indicator for carbon dioxide saturation. As pressure built in the KOH bottle, the solution was displaced through another tube in the septum (F) which was connected to a 50 mL pipette with 0.5 mL graduations (G). Once gas ceased bubbling from the BMP bottle and the KOH solution was equilibrated between the bottle and pipette, the mL of KOH displaced into the pipette was measured, which corresponded to the methane produced from the BMP bottle. The BMP bottle was then disconnected and the pipette was zeroed by placing KOH bottle upright and draining the displaced KOH solution back into the bottle while venting the headspace with a needle through the KOH bottle septum. The BMP bottle was then returned to the incubator.

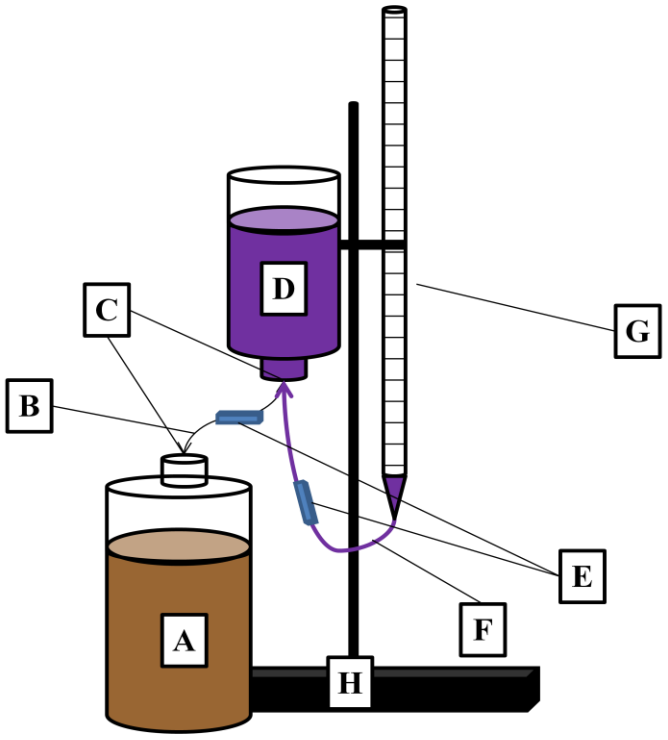


Figure 2-1. Apparatus for measuring methane production through hydraulic displacement. Components are: A) BMP Bottle, B) tube for biogas flow, C) needles in septa, D), 5M KOH with alizarin in 250 mL serum bottle, E) roller clamps for tube closure, F) tube for displaced KOH solution, G) 50mL pipette with 0.5 mL graduations, H) stand.

Gas measurements were taken for 30 days in both BMP assays. All methane production values were standardized to standard temperature and pressure (STP) (0°C and 1 atm) and calculated per g COD loaded basis after subtracting methane production from the inoculum blanks. The percent of substrate COD converted to methane (% COD removal) was calculated based on the stoichiometric COD equivalence of methane (2.86 g COD/L CH_4 @ STP) as a fraction of TCOD loaded. Total COD, SCOD, pH, and conductivity were measured on the digestate from each bottle in both BMP assays after 30 days. Alkalinity was also measured on the digestate from each bottle in the reduced-loading-rate assay after 30 days.

Statistical Analysis

Cumulative methane production was fit to a first-order kinetic model using non-linear fitting.

$$CH_4 = CH_{4f} * (1 - e^{(-k*t)})$$

Where:

- CH_4= cumulative methane production at time t (mL/g COD loaded @STP)
- CH_{4f}=ultimate cumulative methane production (mL/g COD loaded @STP)
- k=kinetic rate constant (day^{-1})

The solver application in Microsoft Excel 2007 was utilized to solve for CH_{4f} and k by minimizing the residual sum of squares of triplicate data points. Standard error of each parameter was calculated using the square root of the variance. A Student's t-test (α=0.05) was used to determine significant differences between mean values of parameters.

Physiochemical Parameters

Total Chemical Oxygen Demand

Total COD was measured using a modification to standard methods (APHA, 2005). Triplicate 25 g (wet weight (ww)) mixed, representative samples of food waste were obtained. The samples were fully homogenized using a 360 mL stainless steel blender (Waring,

Torrington, CT) for 1 minute on high speed. Deionized water was added during blending to facilitate homogenization. The entire homogenized samples were diluted to 500 mL with DI water using volumetric flasks. The diluted samples were poured into 600 mL glass beakers and fully homogenized with magnetic stir bars. Using a transfer pipette and volumetric flask, a 25 mL aliquot was obtained from each sample and further diluted to 250 mL with DI water in a 250 mL volumetric flask for a final dilution of 1 g food waste/200 mL. A 1 mL aliquot of the 1/200 diluted samples and 1 mL DI water were added to Hach COD reagent tubes (HR 20-1500 mg COD/L). Tubes were digested for 2 h at 150°C in a Hach Model 45600 COD reactor (Hach Company, Loveland, CO). Digested tubes were read on a Hach 890 colorimeter (Hach Company, Loveland, CO).

Total COD on inocula and digestate following BMP assays was measured by diluting a 5 mL mixed aliquot to the requisite dilution within the range of the COD reagent tubes. Two mL of diluted aliquot were then placed in the COD tube and analyzed as described above.

Soluble Chemical Oxygen Demand

To measure soluble chemical oxygen demand (SCOD), samples were completely mixed with a magnetic stir bar to obtain representative aliquots. A 20 mL aliquot was transferred into a 25 mL glass centrifuge tube using a transfer pipette. Tubes were promptly placed into a refrigerated water bath (Refrigerated Bath Model 90, Thermo Fisher Scientific, Waltham, MA) at 4°C to quench enzyme activity and prevent further metabolism. Aliquots were then centrifuged at 12,000 rpm for 30 minutes in a refrigerated centrifuge (Sorvall ® RC-5B Refrigerated Superspeed Centrifuge, DuPont Instruments, Wilmington, DE) at 4°C. After centrifuging, the supernatant of each aliquot was filtered through a 0.45 µm nylon syringe filter (Thermo Fisher Scientific, Waltham, MA) using a 3 mL syringe. Filtered supernatant was diluted to required concentration with DI water. Two mL of diluted supernatant were placed into

a Hach COD reagent tube (HR 20-1500 mg COD/L) and digested at 150°C for 2 h in a Hach Model 45600 COD reactor (Hach Company, Loveland, CO). Digested tubes were read on a Hach 890 colorimeter (Hach Company, Loveland, CO).

To measure endogenous SCOD of food waste, triplicate 25 g (ww) mixed, representative samples of food waste were obtained. The samples were fully homogenized using a 360 mL stainless steel blender (Waring, Torrington, CT) for 1 minute on high speed. Deionized water was added during blending to facilitate homogenization. Blended food waste was diluted to 250 mL with DI water. A 20 mL aliquot was measured for SCOD as described above.

Total Solids and Volatile Solids

Total solids (TS) and volatile solids (VS) were measured according to standard methods (APHA, 2005). Triplicate 100 g mixed, representative samples of food waste were weighed into pre-ashed, pre-weighed 200 mL disposable aluminum dishes. Samples were dried at 103°C in a drying oven (Precision Model STG 80, Thermo Fisher Scientific, Waltham, MA) for 24 h. Dried samples were placed in a desiccator to cool to room temperature, then weighed to record TS. To measure volatile solids (VS), dried samples were ashed for 2 h in an ashing furnace (Thermolyne 30400, Thermo Fisher Scientific, Waltham, MA) at 550°C. Ashed samples were placed in a dessicator to cool to room temperature and then weighed. Ash weight was subtracted from TS to calculate VS.

pH

An Accumet Model 10 pH meter (Thermo Fisher Scientific, Waltham, MA) using a Ross Sure Flow combination electrode was used for measuring pH. Samples were gently mixed with a magnetic stir bar during measurement to reduce CO_2 evolution. Sample temperature was accounted for when measuring pH.

Total Nitrogen and Total Phosphorus

Total nitrogen (TN) and total phosphorus (TP) were measured by blending a 25 g representative sample of food waste in a 360 mL stainless steel blender for 1 minute. Triplicate 0.25 g representative subsamples of the blended food waste were weighed onto 1 KimWipe tissue (1 ply, 11 x 21 cm). Samples, including KimWipe, were digested using a modification of the aluminum block digestion procedure of Gallaher et al. (1975). Catalyst used was 1.5 g of 9:1 K_2SO_4:$CuSO_4$, and digestion was conducted for at least 4 h at 375°C using 6 ml of H_2SO_4 and 2 ml H_2O_2. Nitrogen and phosphorus in the digestate were measured by semiautomated colorimetry (Hambleton, 1977). A blank KimWipe was also measured to correct for the TN and TP in the tissue.

Alkalinity

Alkalinity was measured using a modification to standard methods (APHA, 2005). A 25 g (ww) sample of food waste was mixed with 25 mL DI water. The food waste solution was then titrated with 0.12 N H_2SO_4 (standardized with 0.05 N $NaCO_3$) while mixing with a magnetic stir bar until a pH of 4.5 was reached. Alkalinity was calculated using the following formula.

$$Alkalinity\ (mg\ CaCO_3\ eq./kg\ food\ waste\ (ww)) = \frac{mL\ H_2SO_4\ used * 0.12 * 50{,}000}{(25\ g\ food\ waste)}$$

Alkalinity on digestate from BMP assays was measured by titrating a 50 mL mixed aliquot using the procedure described above. Alkalinity was calculated using the following formula.

$$Alkalinity\ (mg\ CaCO_3\ eq./L) = \frac{mL\ H_2SO_4\ used * 0.12 * 50{,}000}{(50\ mL\ aliquot)}$$

Conductivity

Conductivity was measured on an Accumet Model 30 conductivity meter (Thermo Fisher Scientific, Waltham, MA). The meter was standardized with 0.01M KCl. To measure conductivity of food waste, 25 g (ww) of food waste was diluted with 25 mL DI and mixed using a magnetic stir bar.

Organic Acid and Sugar Analysis

Organic acids and sugars were measured by high-pressure liquid chromatography (HPLC) using an HP 1090 Series II chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a Bio-Rad Aminex HPX-87H ion exclusion column (45°C; solvent phase, 4 mM H_2SO_4; flow rate, 0.5 ml min^{-1}; injection volume, 10 μl) and dual detectors (refractive index monitor and UV detector at 210 nm). One mL of supernatant samples from SCOD analysis was filtered using a 0.2 μm nylon syringe filter. Ten μL of 0.1 N sulfuric acid were added to 1 mL samples. Samples were analyzed with standards for glucose, xylose, lactic acid, succinic acid, formic acid, acetic acid, propionic acid, butyric acid, and ethanol.

CHAPTER 3
EFFECT OF MECHANICAL PRETREATMENT ON SOLUBILIZATION OF FOOD WASTE

The anaerobic digestion of high particulate feedstock, such as food waste, is considered to be limited by the rate of solubilization (Eastman and Ferguson 1981, Palmowski and Muller 2003, Wang et al. 2006, Izumi et al. 2010). Increasing the solubilization of food waste through pretreatment is proposed as a solution to increase the rate and extent of food waste anaerobic digestion. Pretreatment methods examined in literature include thermal, freezing/thawing, enzymatic, and mechanical. However the methods proposed in literature are energy and resource intensive and are impractical for commercial-scale utilization. Therefore, it is necessary to study the enhancement of food waste solubilization kinetics through implementing low-tech, practical pretreatment methods. Three mechanical pretreatment methods were examined in this study: a manual meat grinder (with two different plate sizes) and an in-sink food disposer. Different pretreatment methods were tested to discern any measureable differences between these methods. In order to study the effect of pretreatment on food waste solubilization, a series of solubilization assays were conducted comparing pretreated food waste to intact food waste. Solubilization is defined in the present study as the sum of the release of endogenous soluble organic material and the enzymatic hydrolysis of particulate material. By placing food waste in water, the release of the endogenous soluble organic material was able to be measured. The assay measured the rate and extent that the soluble material is released into the aqueous solution. This release can be a result of both physical cell rupture and tissue disruption or hydraulic leaching. Enzymatic hydrolysis was measured by adding either a commercial hydrolytic enzyme powder or a microbial inoculum to the food waste. Hydrolysis was then calculated by subtracting the release of endogenous soluble organic material from the total solubilization. Solubilization in the assays was measured as soluble chemical oxygen demand (SCOD) and was

normalized as a fraction of total COD (TCOD) to account for natural variation in the standard food waste. Solubilization is presented as the COD soluble fraction (CODsf) and is divided into solubilization through endogenous SCOD release (CODesf) and solubilization though enzymatic hydrolysis (CODhsf). In the assays, buffering and dilution were required because preliminary solubilization assays without buffering or dilution resulted in sharp decreases in pH (Figure 3-1), which inhibited enzymatic and microbial activity. This chapter discusses the results of three solubilization assays: 1) an endogenous solubilization experiment, 2) a commercial enzymes experiment, and 3) a microbial inoculum experiment.

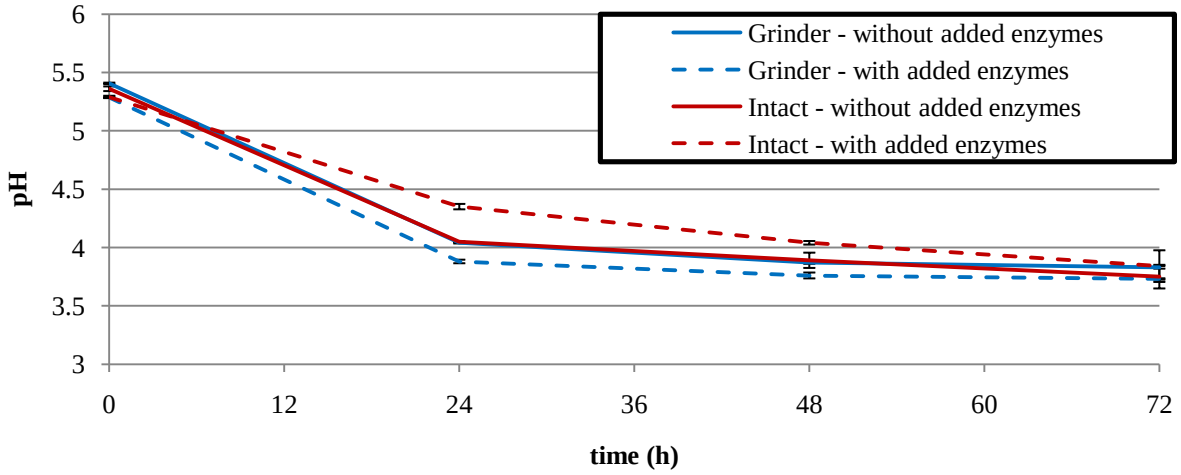


Figure 3-1. Acidification of food waste under unbuffered, undiluted conditions.
Note: food waste loading rate was 500 g (ww)/L (175 g COD/L)

Microscopy of Pretreated Food Waste

Intact and pretreated food waste was observed microscopically to elucidate structural changes in food waste through mechanical pretreatment that would favor increased solubilization. Figures 3-2 to 3-5 show photomicrographs of intact (A) and pretreated (B) standard food waste components. The photomicrographs show that pretreatment leads to significant structural changes that result in both cell rupture, which releases intracellular content, and tissue disruption, which causes looser structure to facilitate enzymatic hydrolysis. The

solubilization assays were conducted to quantify the effects of this structure change on solubilization.

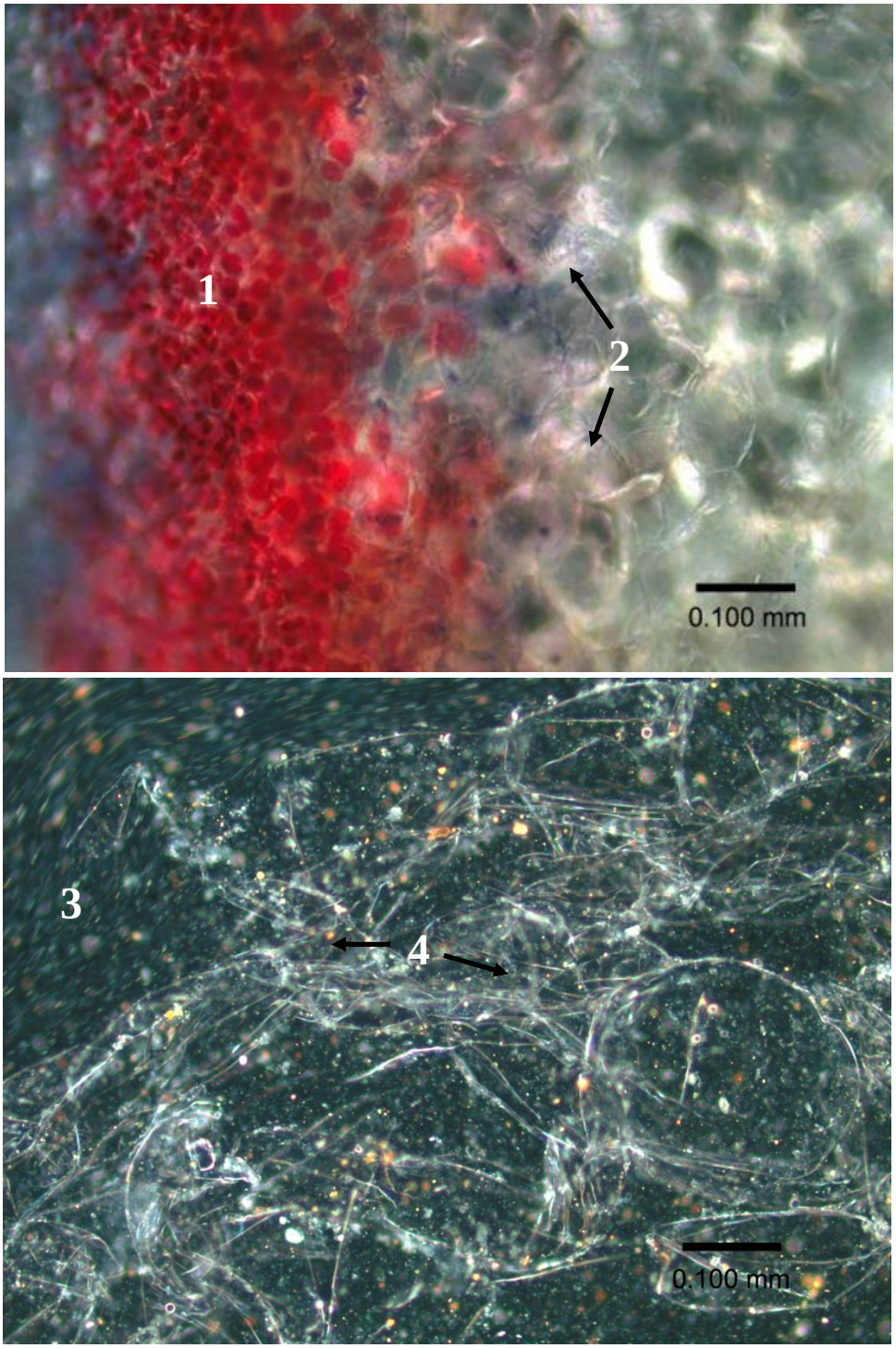
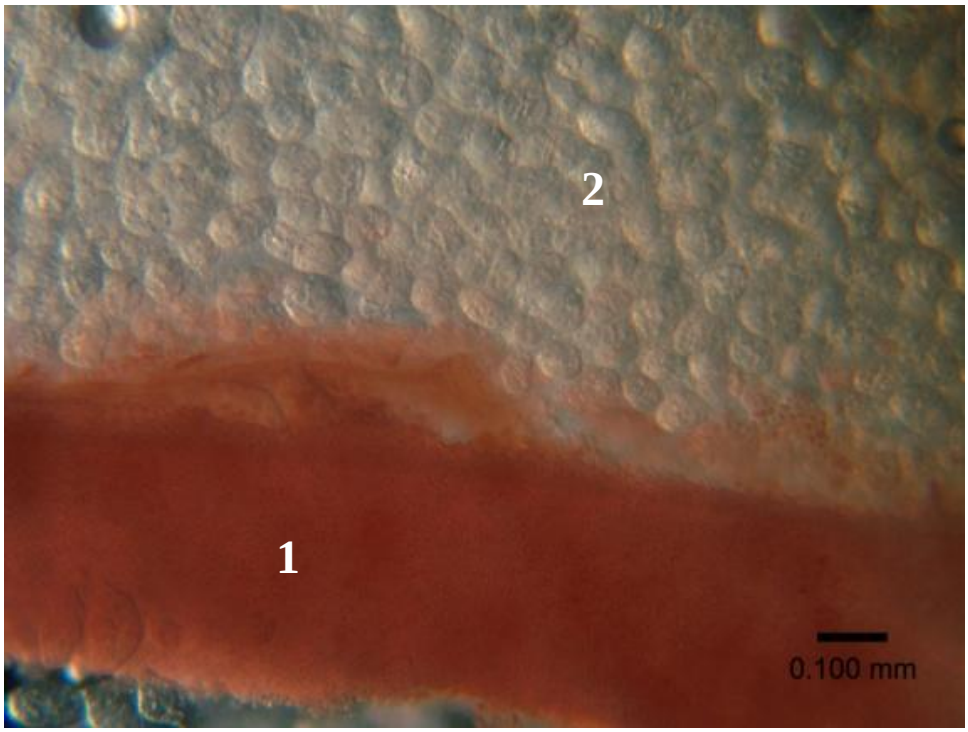


Figure 3-2. Photomicrographs of apple.
A) Intact apple with intact skin tissue (1) and intact cells (2). B) Pretreated apple with released intracellular material (3) and cell wall debris (4).

A



B

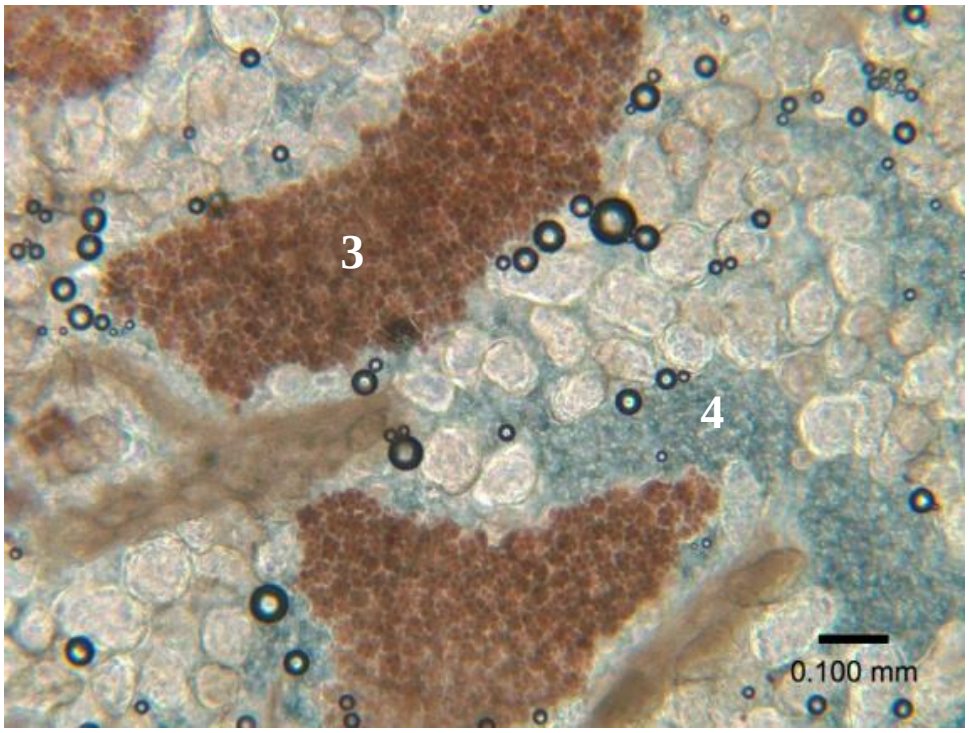
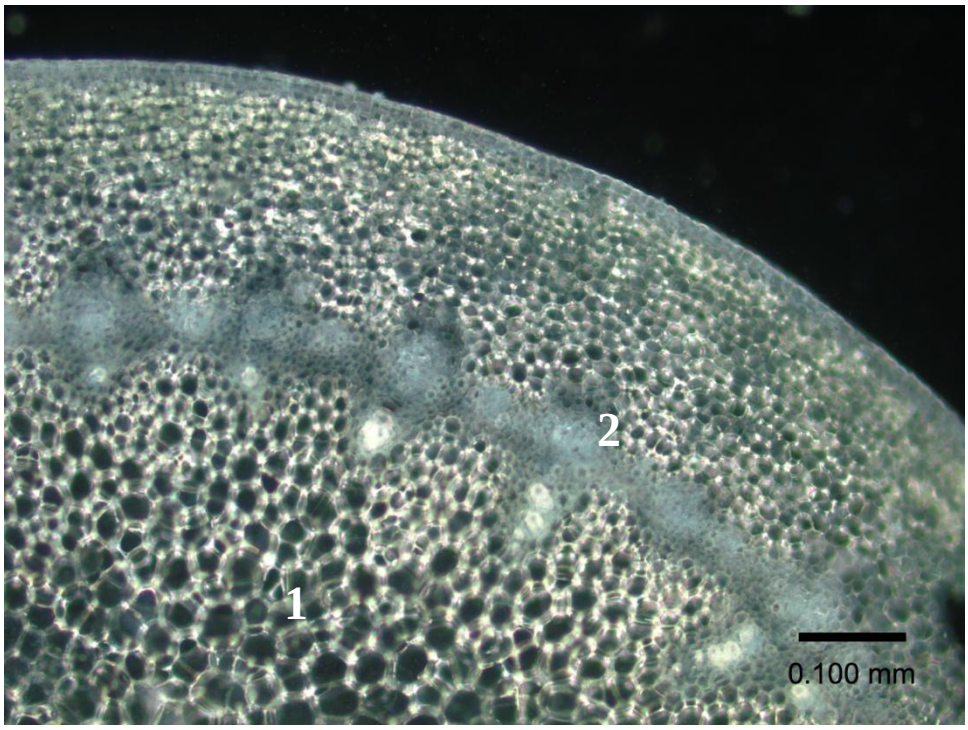


Figure 3-3. Photomicrographs of bean.
A) Intact bean with intact skin tissue (1) and intact cells (2). B) Pretreated bean with fragmented skin tissue (3) and released intracellular material (4).

A



B

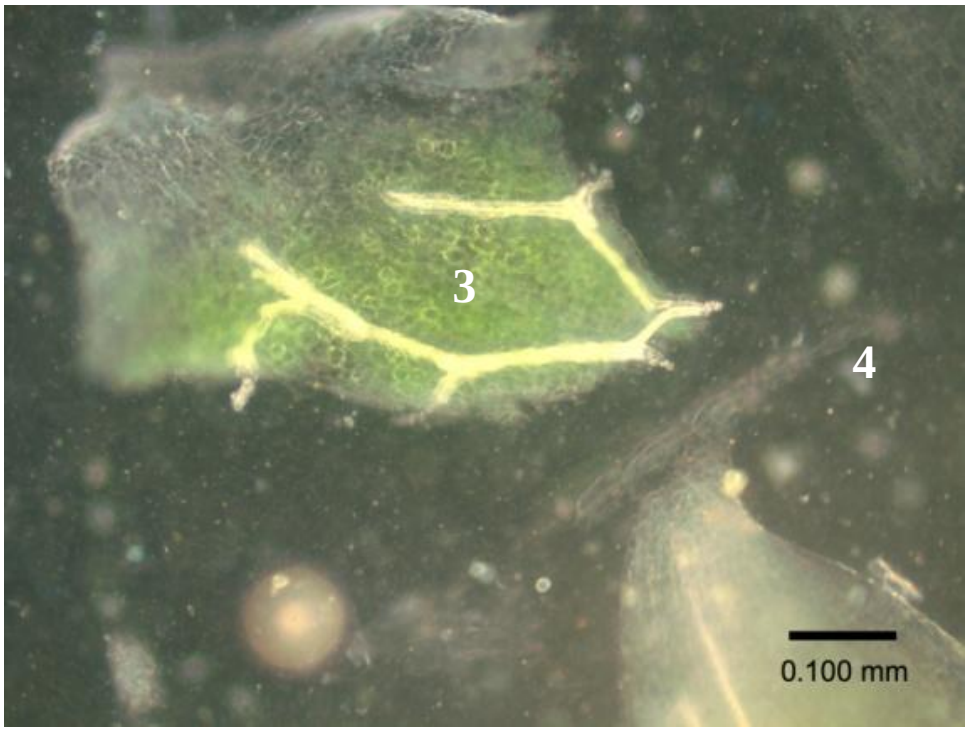
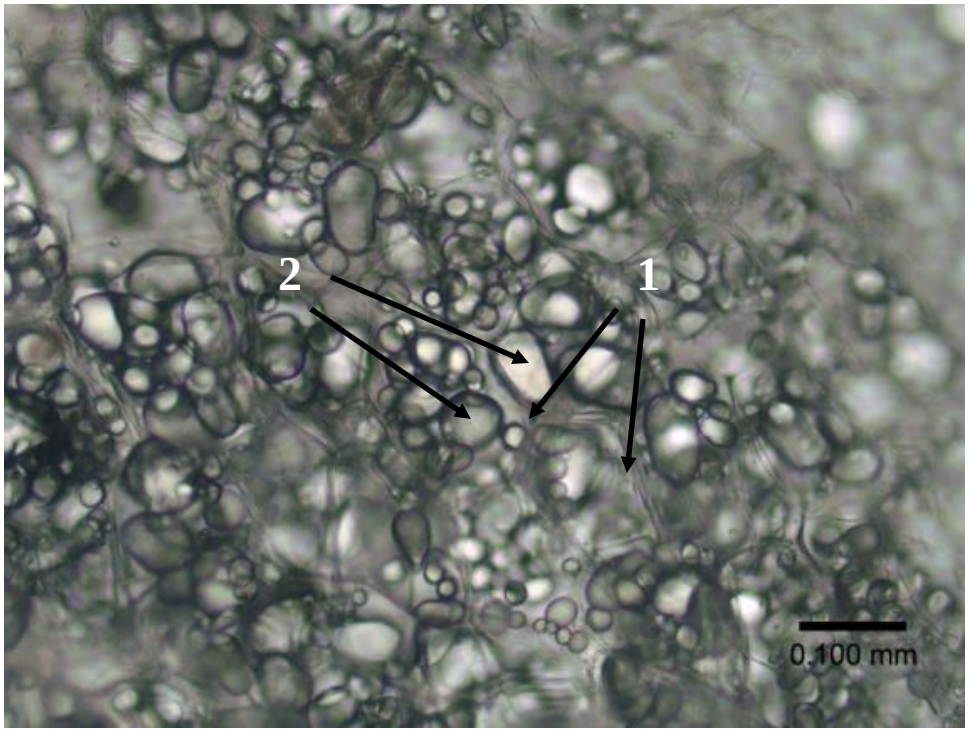


Figure 3-4. Photomicrographs of broccoli.
A) Transverse section of intact broccoli stem with intact vascular tissue and (1) and intact cells (2). B) Pretreated broccoli with fragmented stem tissue (3) and released intracellular material (4).

A



B

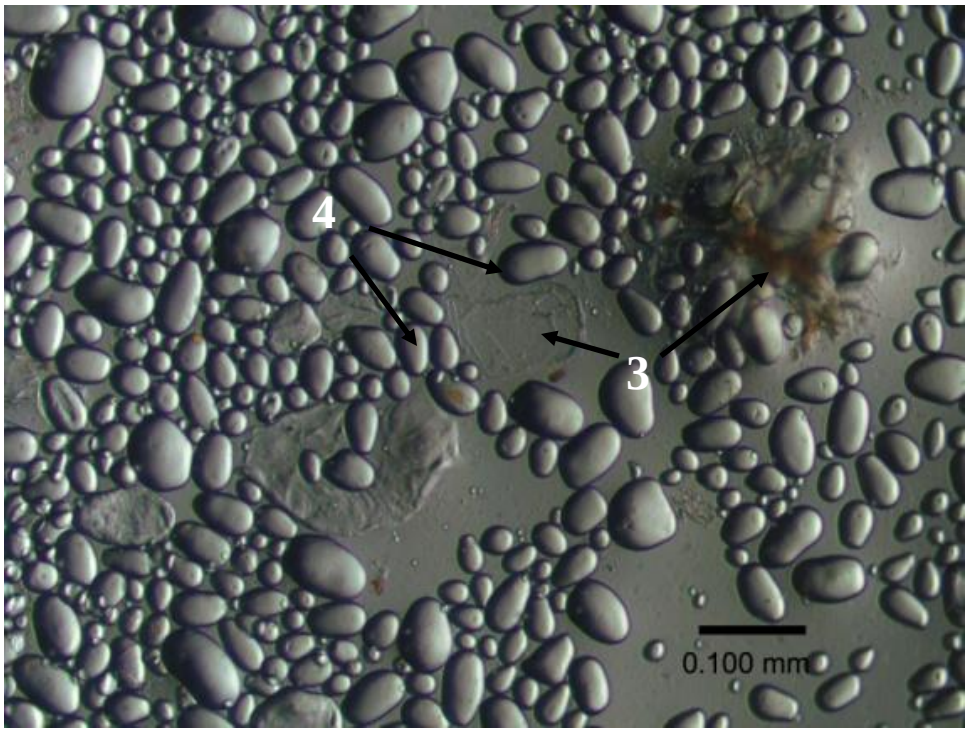


Figure 3-5. Photomicrographs of potato.
A) Intact potato with intact cells (1) and intracellular starch (2). B) Pretreated potato with cell debris (3) and free starch (4).

Endogenous Solubilization Assay

An endogenous solubilization assay was conducted by adding intact and pretreated food waste to water. The purpose of this assay was to determine release of the endogenous SCOD of pretreated and intact food waste. When measuring the physiochemical parameter of the standard food waste, it was determined that the endogenous soluble chemical oxygen demand (SCOD) was 87.9 mg/kg (ww) or 25% soluble fraction of COD (CODsf) (Table 2-3). This is the approximate maximum of endogenous SCOD release.

Twenty-four Hour Solubilization

The assay was conducted for 24 h with SCOD and pH measured at 0, 1, 2, 4, 6, 8, 12 and 24 h (for data analysis purposes 0.1 h is the nominal time allocated for pretreatment). Figure 3-6 presents the triplicate SCOD measurements of intact and pretreated food waste. In all treatments, except intact food waste, solubilization appeared to plateau prior to 8 h, and solubilization decreased at 12 and 24 h. This decrease was likely due to microbial assimilation of SCOD. Microbial assimilation was suggested by the decrease in pH at 12 and 24 h (Figure 3-7), which was a result of bacterial fermentation and acidogenesis. Additionally, organic acid and sugar analysis of the samples confirmed that sugars were almost entirely consumed by 12 h (Figures 3-8 and 3-9). At 1 h, 15% of SCOD of pretreated food waste was glucose but was below detection by 12 h. For further data analysis, the 12 and 24 h data points were disregarded because the bacterial assimilation of SCOD confounds any further increases in solubilization. Figure 3-10 shows the mean solubilization of food waste over the first 8 h. Because there was minimal enzymatic hydrolysis in this assay, the total solubilization (CODsf) represented solubilization through the release of endogenous SCOD (CODesf) from the food waste.

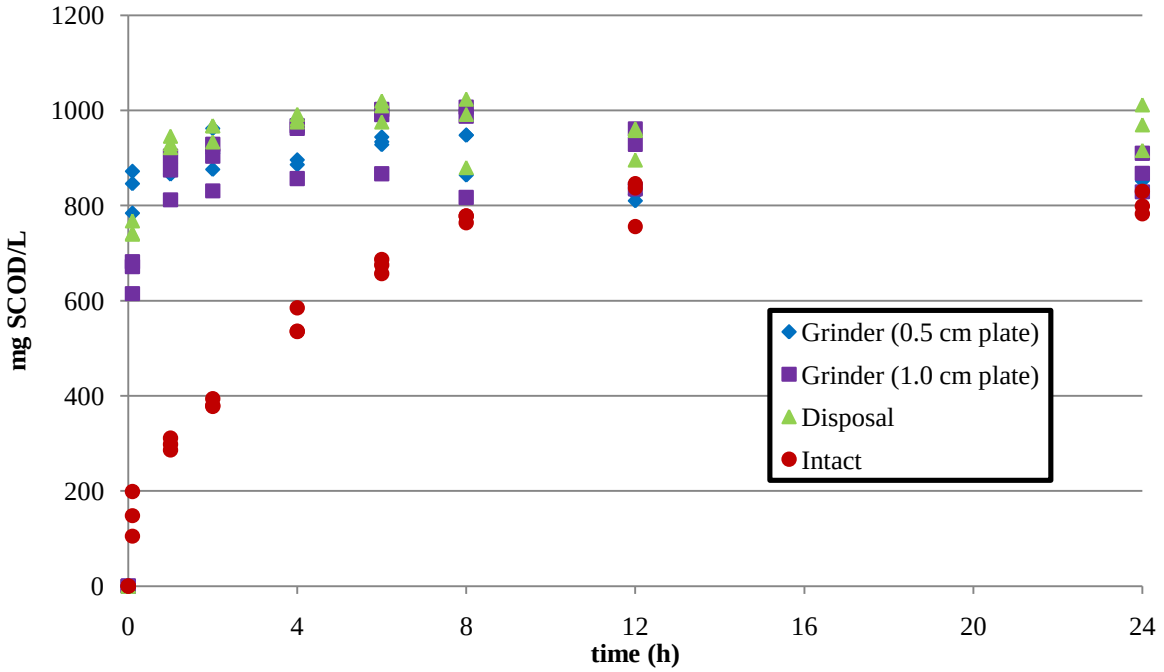


Figure 3-6. Soluble chemical oxygen demand in the endogenous solubilization assay.
Note: Total COD of grinder (0.5 cm plate), grinder (1.0 cm plate), disposer, and intact food waste is 3284, 3368, 3556, 3688 mg/L, respectively.

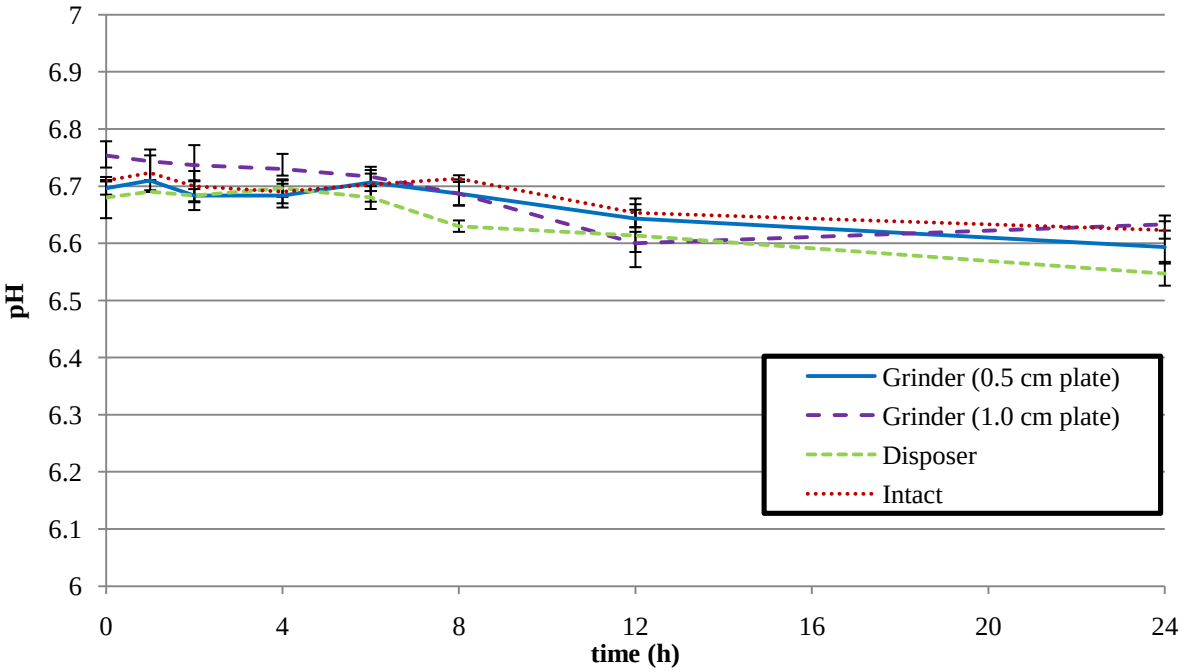


Figure 3-7. Mean pH for the endogenous solubilization assay. Error bars represent standard deviation.

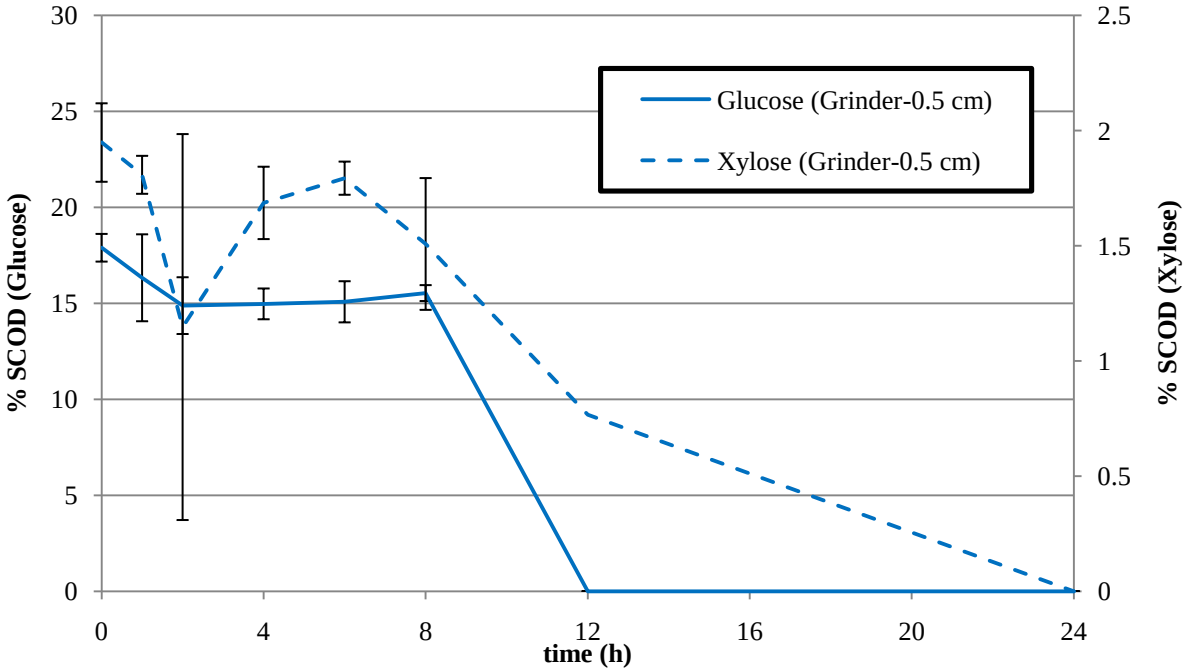


Figure 3-8. Sugars as a percent of SCOD for pretreated food waste (grinder-0.5 cm plate) in the endogenous solubilization assay. Error bars represent standard deviation.

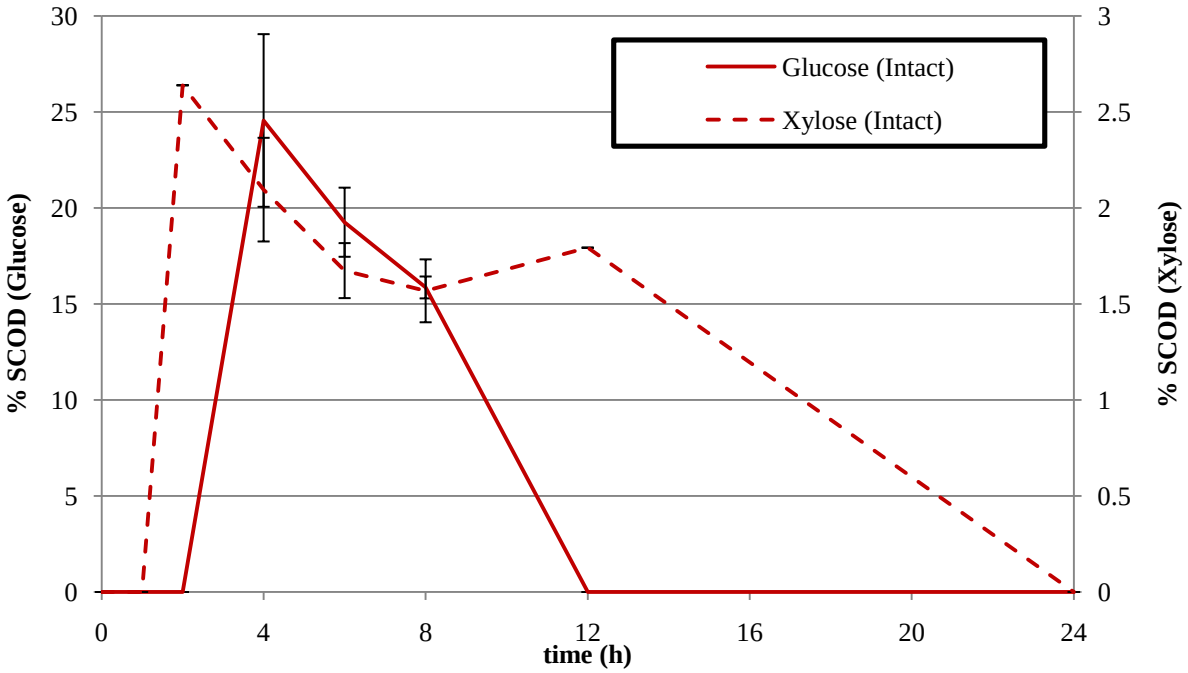


Figure 3-9. Sugars as a percent of SCOD for intact food waste in the endogenous solubilization assay. Error bars represent standard deviation.

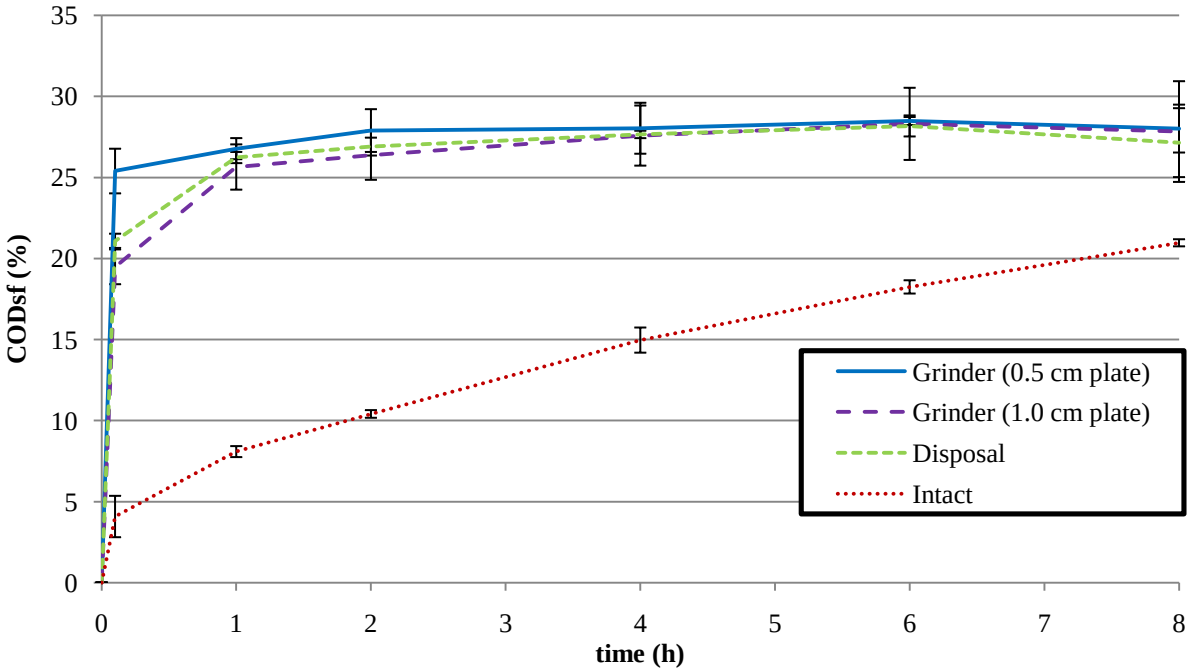


Figure 3-10. Mean solubilization of food waste in the endogenous solubilization assay. Error bars represent standard deviation.

Endogenous Solubilization Kinetics

The release of endogenous SCOD was fit to a first order kinetic equation with the kinetic rate constant (k) and final endogenous soluble fraction ($CODesf_f$) as fitted parameters.

Estimates of parameters

Table 3-1. Estimated parameters and 8 h endogenous solubilization in endogenous solubilization assay

	k (h^{-1})		$CODesf_f$ (%)		$CODesf_8$ (%)
Treatment	Estimate[a]	SE[b]	Estimate[a]	SE[b]	
Grinder (0.5 cm plate)	24.328 X	2.564	27.840 X	0.260	27.840
Grinder (1.0 cm plate)	12.654 Y	1.356	27.146 XY	0.442	27.146
Disposer	14.907 Y	0.912	27.222 Y	0.236	27.222
Intact	0.364 Z	0.028	21.089 Z	0.609	19.943

a: Different letters in the same column indicate significant difference (α=0.05)
b: Standard error

Table 3-1 shows estimates of the fitted parameters and 8 h CODesf for each treatment in the assay. All three pretreatment methods showed significantly higher kinetic rate constants (k) and $CODesf_f$, than intact food waste. The meat grinder with the 0.5 cm plate showed a

significantly higher k than other two pretreatments and a significantly higher $CODesf_f$ than the disposer at α=0.05; at α=0.01 all three pretreatments had a similar $CODesf_f$.

Fitted curves.

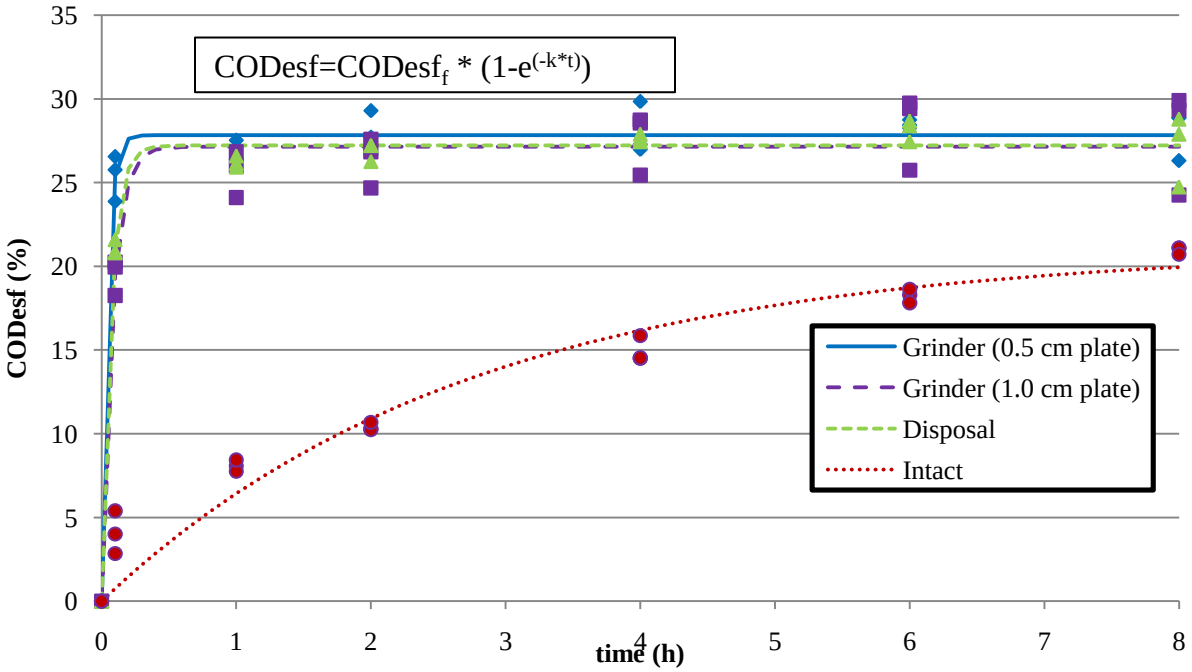


Figure 3-11. Fitted endogenous solubilization curves for the endogenous solubilization assay

The fitted curves with measured data points are presented in Figure 3-11. All three pretreatment methods showed much faster solubilization than intact food waste. The pretreatment immediately released the endogenous SCOD of the food waste. The meat grinder (0.5 cm plate) showed more immediate release than either other pretreatment; however this difference was negligible as the difference was only within the first hour. All three pretreatments released the full complement of endogenous SCOD (measured at approximately 25%) (Table 2-3) immediately through the macerating action of pretreatment alone. Intact food waste, however, showed a much slower release of endogenous SCOD (k=0.36 h^{-1}). The release of endogenous SCOD from intact food waste occurred through hydraulic leaching, which was a

slow process compared to the immediate expulsion through pretreatment. After 8 h, the full complement of endogenous SCOD was not released from the intact food waste.

Commercial Enzyme Assay

For particulate feedstocks, the majority of solubilization occurs as a result of enzymatic hydrolysis (Eastman and Ferguson 1981). Pretreatment can increase the availability of food waste to these enzymes through decreased particle size and looser physical structure. To measure the enzymatic availability of food waste, hydrolytic enzymes were included in a solubilization assay. A commercial hydrolytic enzyme powder was selected to represent the hydrolytic enzymes produced in an anaerobic digester. The commercial enzymes were applied in an excess amount in the assay (0.1 g/g food waste (ww)), so that the enzyme quantity would not be limited. This allows the differences in substrate availability to be distinguished

Twenty-four Hour Solubilization

The assay was conducted for 24 h with SCOD and pH measured at 0, 1, 2, 4, 6, 8, 12 and 24 h (for data analysis purposes 0.1 h is the nominal time allocated for pretreatment). Figure 3-12 presents the SCOD of intact food waste and food waste pretreated with meat grinder (0.5 cm plate and 1.0 cm plate) and food waste disposer. As in the endogenous solubilization assay, solubilization appeared to plateau within 6-8 h and decrease at 12 and 24 h. The decrease was likely due to microbial assimilation of SCOD, which was suggested by the drop in pH (Figure 3-13), consumption of sugars (Figures 3-14 and 3-15), and production of organic acids (Figures 3-16 and 3-17). For the first 8 hours in the assay, glucose was approximately 50% of the SCOD of pretreated food waste, and was 10% at 12h. Formic, acetic, and succinic acids and ethanol were at detectable ranges by 24 h, which indicated that SCOD had been consumed for acidogenesis. For further data analysis, the 12 and 24 h data points were disregarded because the bacterial assimilation of SCOD confounds any further increases in solubilization. Figure 3-18 shows the

mean solubilization of food waste in the commercial enzyme assay. All pretreated food waste was approximately 50% solubilized within 2 h, while intact food waste was approximately 23% solubilized at 2 h. By 6 h pretreated food waste reached 60% solubilization and intact food waste reached a mean solubilization of 45% by 6 h. These percentages represented total solubilization, which included both endogenous SCOD released and enzymatic hydrolysis. To assess the kinetics of enzymatic hydrolysis, the endogenous solubilization (Figure 3-6) from the previous experiment was subtracted from the total solubilization in this experiment.

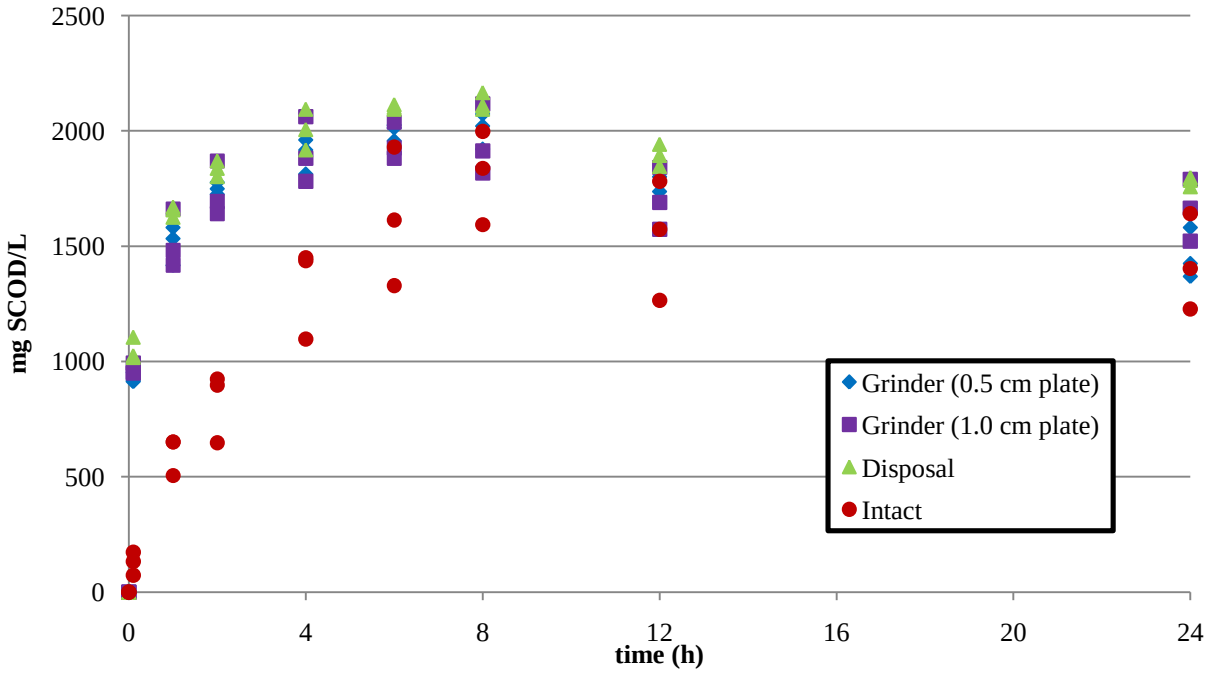


Figure 3-12. Soluble chemical oxygen demand in the commercial enzyme assay.
Note: Total COD of grinder (0.5 cm plate), grinder (1.0 cm plate), disposer, and intact food waste is 3284, 3368, 3556, 3688 mg/L, respectively. Soluble COD from commercial enzyme has been subtracted.

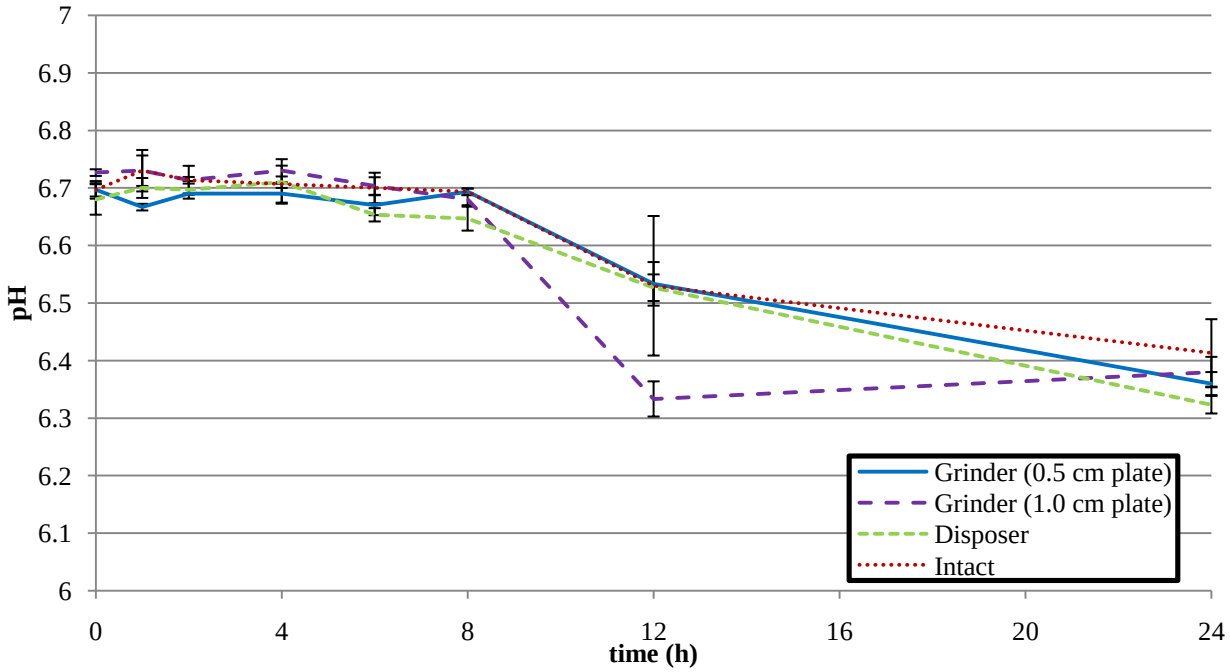


Figure 3-13. Mean pH in the commercial enzyme assay. Error bars represent standard deviation.

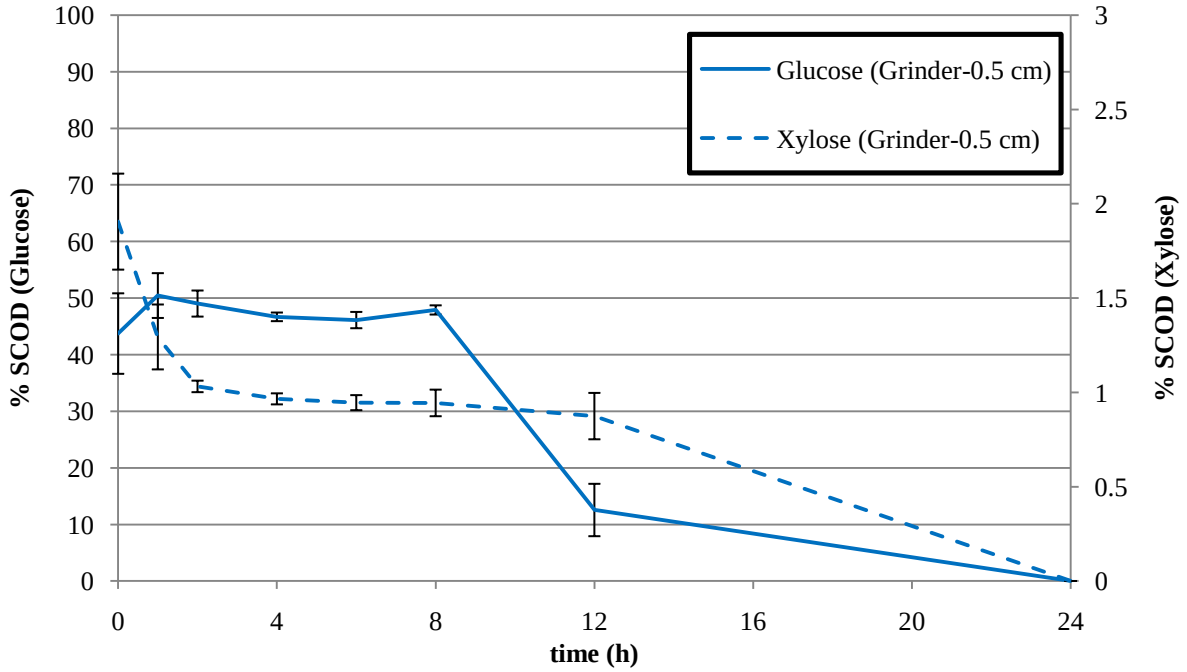


Figure 3-14. Sugars as a percent of SCOD for pretreated food waste (grinder-0.5 cm plate) in the commercial enzyme solubilization assay. Sugars from commercial enzyme have been subtracted.

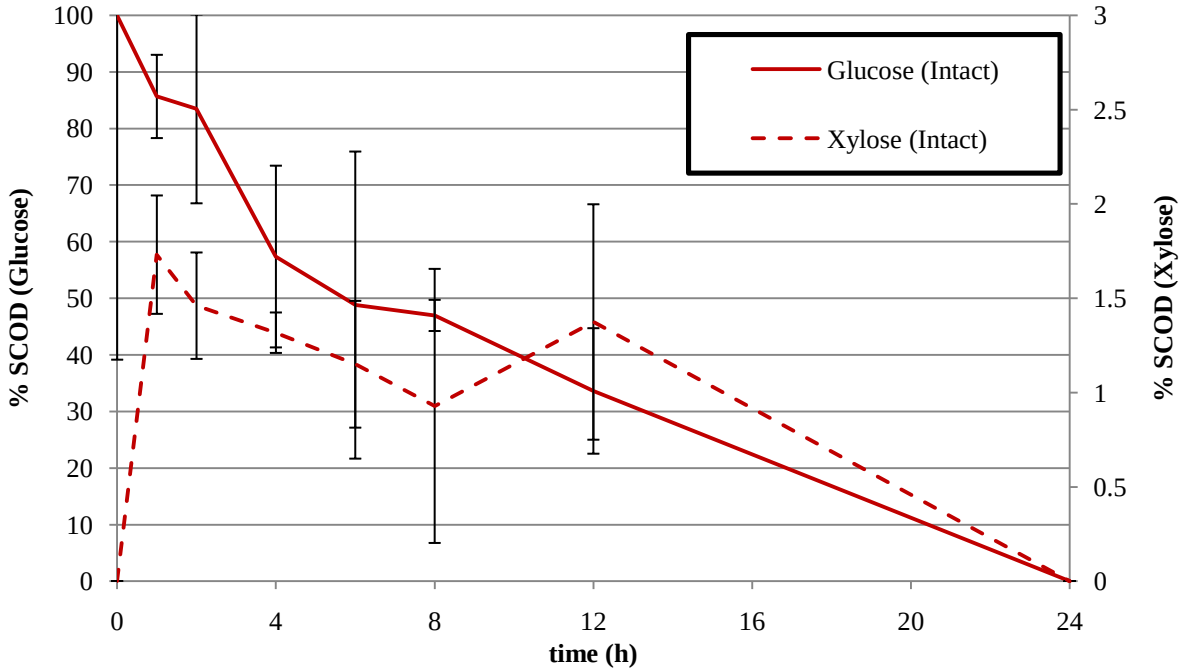


Figure 3-15. Sugars as a percent of SCOD for intact food waste in the commercial enzyme solubilization assay. Sugars from commercial enzyme have been subtracted.

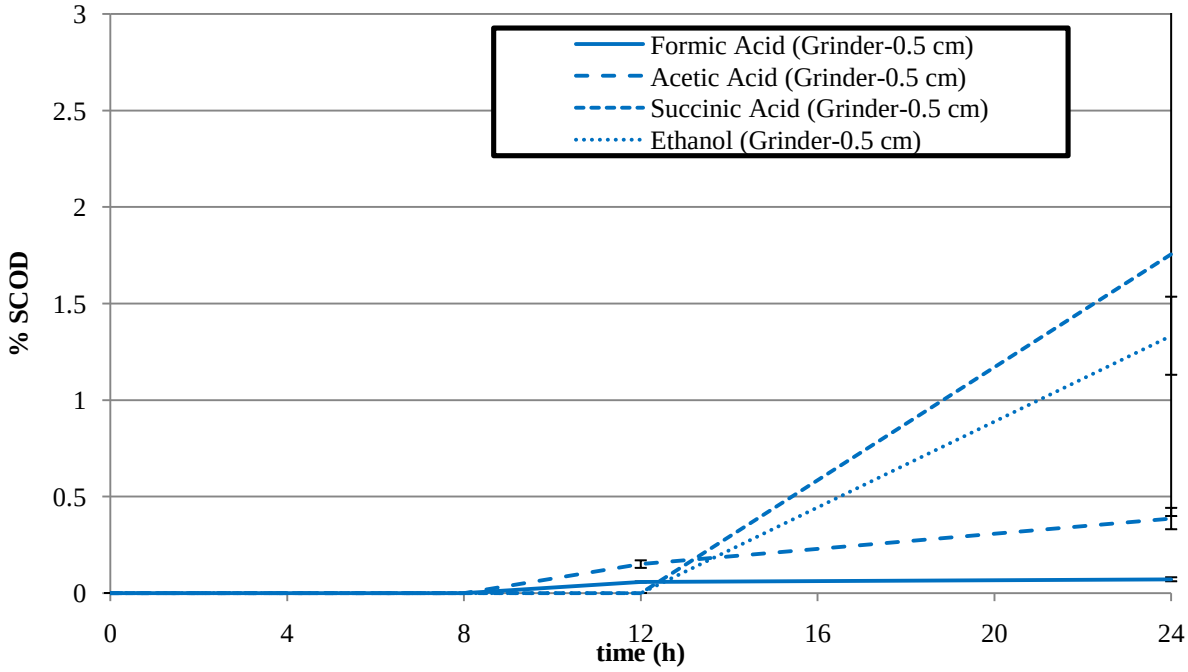


Figure 3-16. Organic acids and ethanol as a percent of SCOD for pretreated food waste (grinder-0.5 cm plate) in the commercial enzyme solubilization assay. Compounds from commercial enzyme have been subtracted.

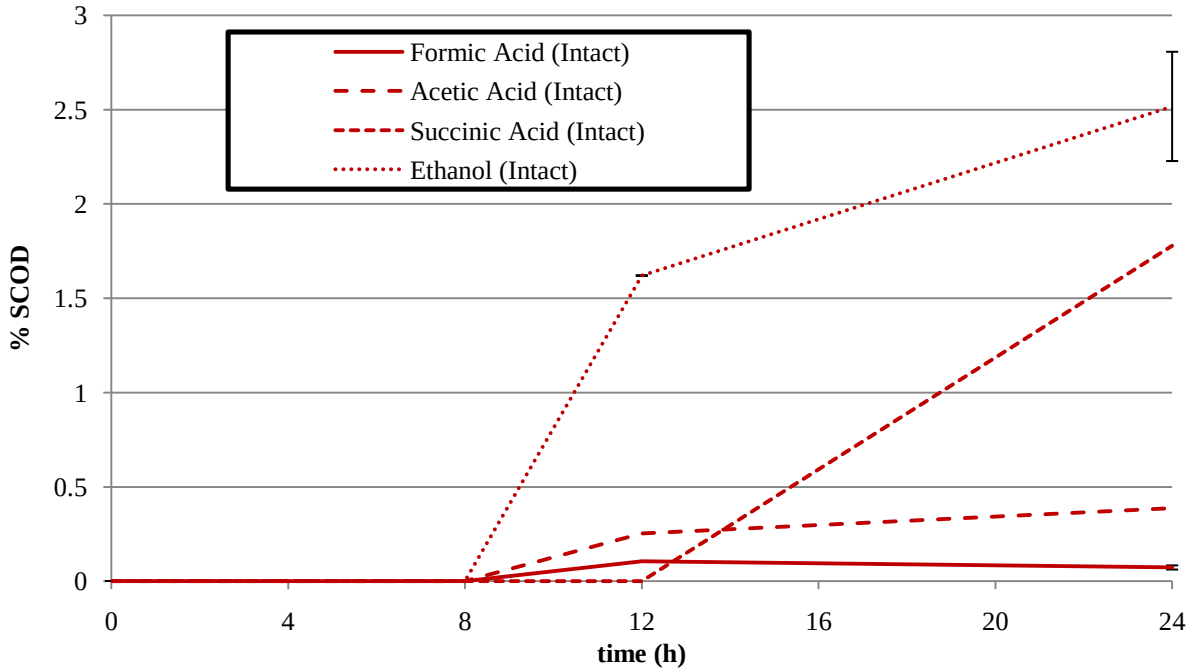


Figure 3-17. Organic acids and ethanol as a percent of SCOD for intact food waste in the commercial enzyme solubilization assay. Compounds from commercial enzyme have been subtracted.

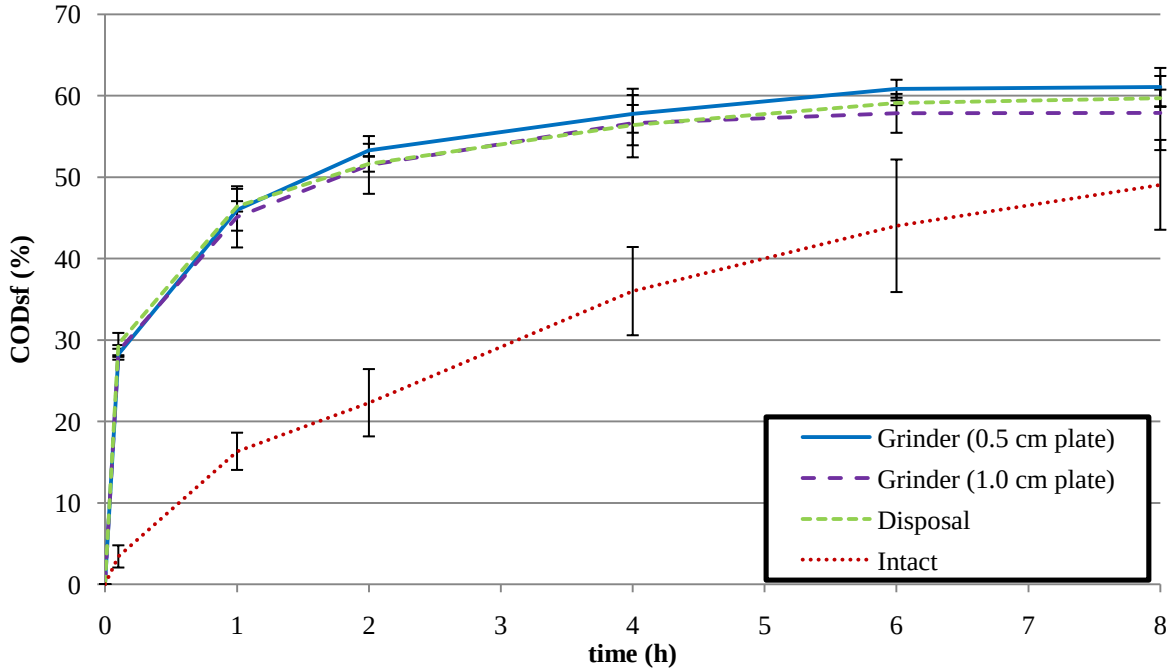


Figure 3-18. Mean solubilization of food waste in the commercial enzyme assay. Error bars represent standard deviation.

Enzymatic Hydrolysis Kinetics

Enzymatic hydrolysis was fit to a first order kinetic equation with the kinetic rate constant (k) and final hydrolyzed soluble fraction ($CODhsf_f$) as fitted parameters.

Estimates of parameters

Table 3-2 shows estimates of the fitted parameters and CODhsf at 8 h for each treatment in the assay. All three pretreatment methods showed significantly higher rate constants than intact food waste. The grinder (0.5 cm plate) had a statistically lower k than either of the other two pretreatments at α=0.05; however at α=0.01, all three pretreatments had a similar k. The grinder (0.5 cm plate) pretreatment and intact food waste had statistically higher $CODhsf_f$ than either of the other two pretreatments a α=0.05; at α=0.01 only the grinder (0.5 cm plate) had a statistically higher $CODhsf_f$ than the grinder (1.0 cm plate).

Table 3-2. Estimated parameters and 8 h hydrolyzed COD for commercial enzyme assay

	k (h^{-1})		$CODhsf_f$ (%)		$CODhsf_8$ (%)
Treatment	Estimate[a]	SE[b]	Estimate[a]	SE[b]	
Grinder (0.5 cm plate)	0.839 X	0.052	32.180 X	0.485	32.141
Grinder (1.0 cm plate)	1.194 Y	0.219	29.228 Y	1.024	29.226
Disposer	1.054 Y	0.130	30.548 Y	0.765	30.541
Intact	0.252 Z	0.027	32.685 X	1.648	28.331

a: Different letters in the same column indicate significant difference (α=0.05)
b: Standard error

Fitted curves

The fitted curves with measured data points are presented in Figure 3-19. All three pretreatments showed much greater hydrolysis rates than intact food waste. At 2 h, enzymatic hydrolysis solubilized over 25% of pretreated food waste and less than 15% of intact food waste. However by 8 hours, approximately 30% of both intact and pretreated food waste was enzymatically hydrolyzed. The increased hydrolysis rates indicated that pretreatment improved the immediate availability for hydrolytic enzymes, due to looser structure and increased surface

area through tissue disruption and cell rupture. The pretreatment of food waste is critical in improving the short-term solubilization of food waste, from both the release of endogenous soluble material and immediate availability to hydrolytic enzymes.

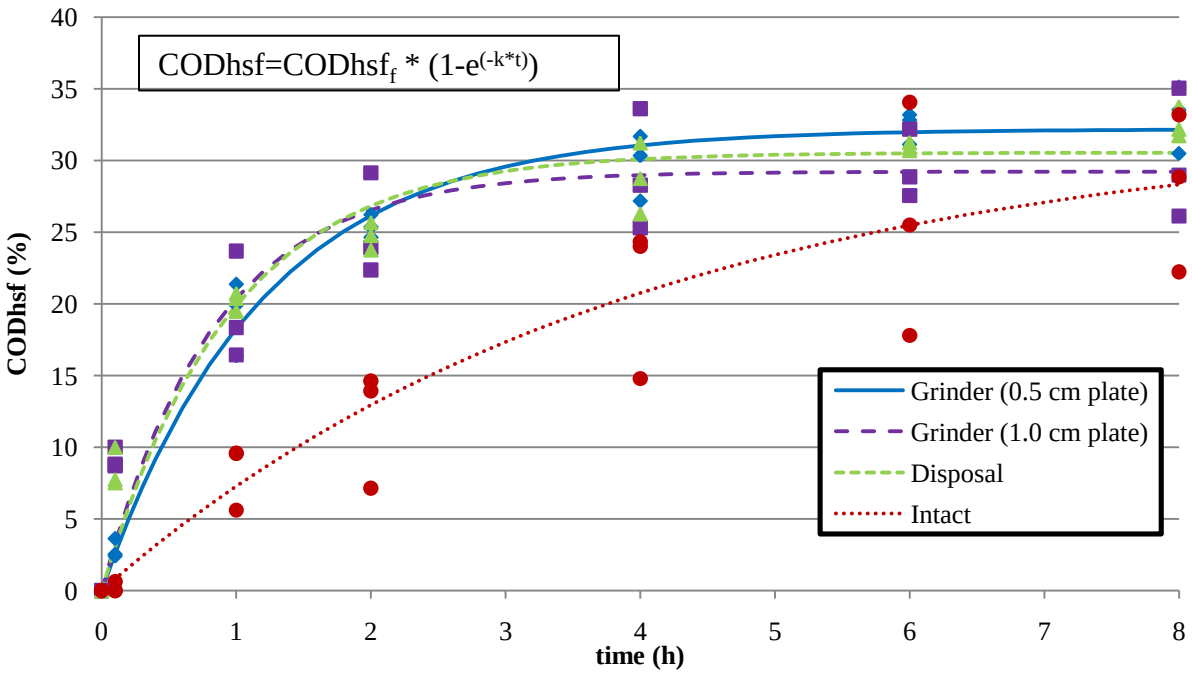


Figure 3-19. Fitted enzymatic hydrolysis curves for the commercial enzyme assay

Microbial Inoculum Assay

The commercial enzyme assay demonstrated food waste hydrolysis kinetics under conditions with excess commercial hydrolytic enzymes. To measure the hydrolysis kinetics of food waste in the presence of microorganisms and enzymes produced by these microorganisms, an experiment was conducted using a microbial inoculum. The purpose of this assay was to simulate the solubilization that would occur in an anaerobic digester by using an inoculum containing a hydrolytic microbial consortium. The microbial inoculum was derived from flushed dairy manure because it contains the microbial consortia, including hydrolytic microorganisms, necessary for anaerobic digestion. Flushed dairy manure was loaded with 10g (ww)/L of pretreated (grinder-0.5 cm plate) food waste and buffered with phosphate buffer (0.5 M at 6.5

pH). The inoculum was incubated for 30 days prior to the assay to allow the hydrolytic consortium to adapt to food waste as a substrate and to the conditions of the assay. In the microbial inoculum assay, pretreatment was represented by the meat grinder with 0.5 cm plate openings. The previous two assays showed there was little difference between pretreatment methods; however, the meat grinder with the 0.5 cm plate did show the most immediate release of endogenous SCOD (Figure 3-10), which represents greater cell and tissue destruction.

Twenty-four Hour Solubilization

The assay was conducted for 24 h with SCOD and pH measured at 0, 1, 2, 4, 6, 8, 12 and 24 h (for data analysis purposes 0.1 h is the nominal time allocated for pretreatment). Figure 3-20 presents the SCOD of intact and pretreated food waste using the microbial inoculum. Unlike the endogenous solubilization and commercial enzyme assays, solubilization did not plateau or decrease at 8 h. The continued increase in solubilization may have been a result of the microorganisms assimilating less SCOD than in the other two assays. In the first two assays, which started at a low microbial population, microorganisms consumed more SCOD as they began to grow and reproduce. However, with the microbial inoculum, there was likely less growth and reproduction, so that there was less SCOD assimilated. The presence of a microbial population throughout the entirety of the microbial inoculum assay was indicated by the steady decrease in pH (Figure 3-21) in the first 8 h followed by pH stabilization from 8 to 24 h. In contrast, the first two assays showed a stable pH from 0 to 8 h followed by a decrease in pH at 12 and 24 h (Figures 3-7 and 3-13). Due to the decreased microbial assimilation of SCOD, solubilization was able to be determined for the full 24 h. As in the commercial enzyme assay, the total solubilization represented both endogenous SCOD release and enzymatic hydrolysis (Figure 3-22). In the microbial inoculum assay, total solubilization reached 52% and 37% for pretreated and intact food waste, respectively. By subtracting the endogenous SCOD release

(Figure 3-6) from total solubilization in this assay, the kinetics of enzymatic hydrolysis were able to be assessed.

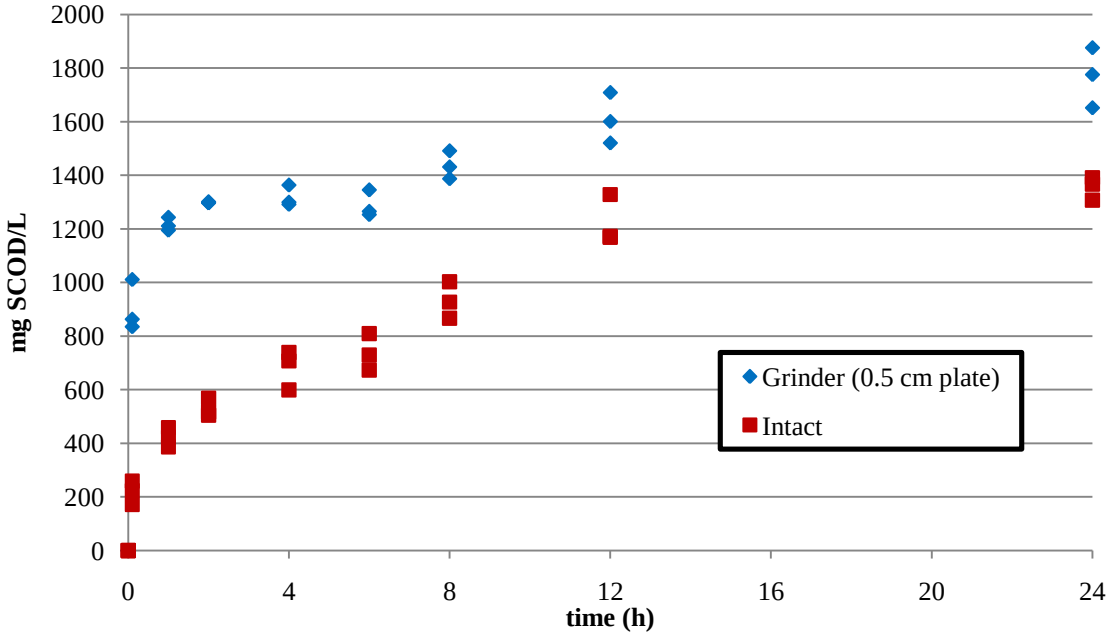


Figure 3-20. Soluble chemical oxygen demand of food waste in the microbial inoculum assay. Note: Total COD of pretreated and intact food waste is 3405 and 3705 mg/L respectively. Soluble COD of microbial inoculum has been subtracted.

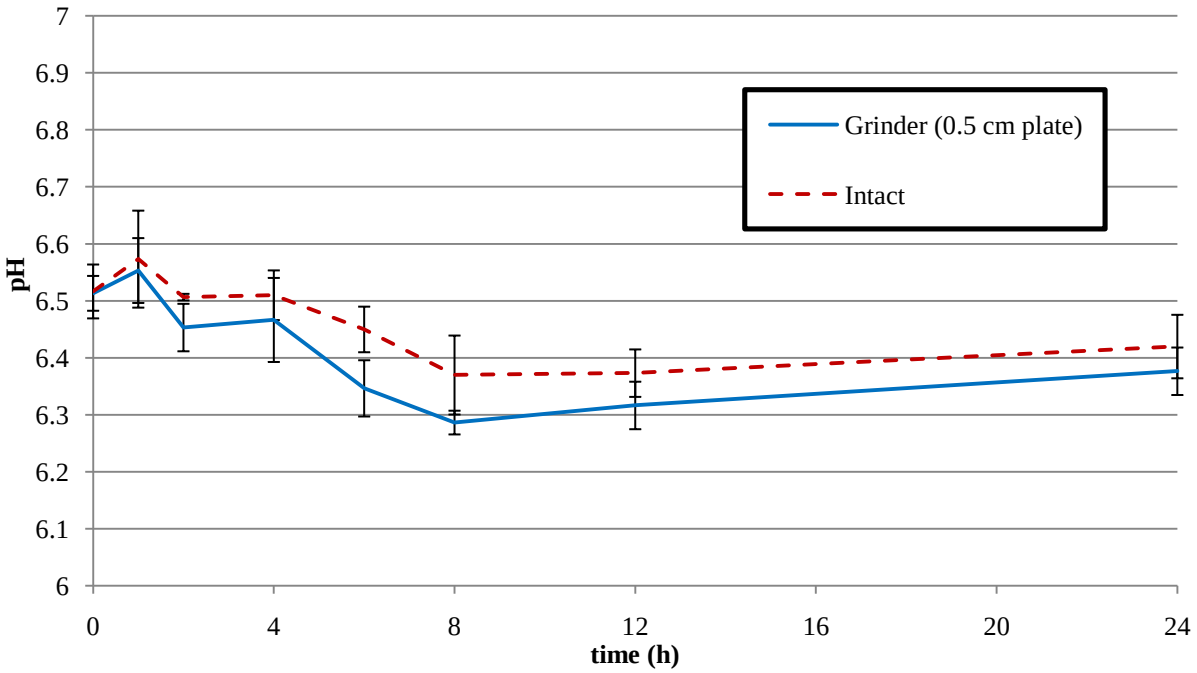


Figure 3-21. Mean pH for the microbial inoculum assay. Error bars represent standard deviation.

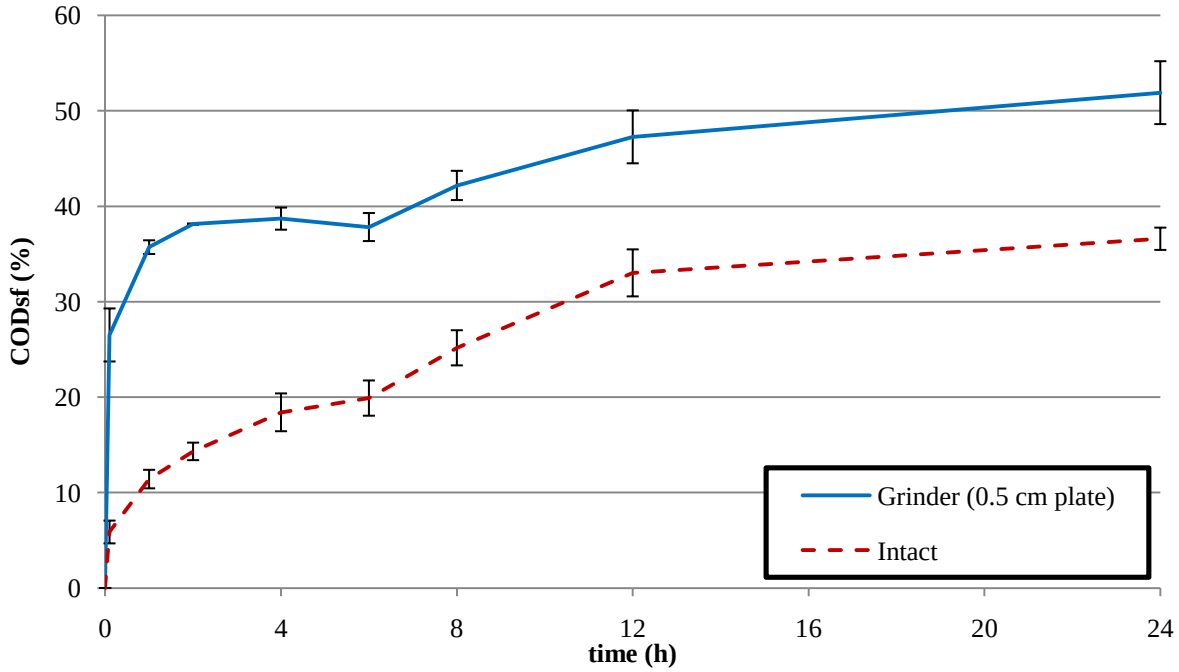


Figure 3-22. Mean solubilization in microbial inoculum assay. Error bars represent standard deviation.

Enzymatic Hydrolysis Kinetics

A noticeable difference between the microbial inoculum assay and the commercial enzyme assay was that enzymatic hydrolysis appeared to have biphasic kinetics. For both intact and pretreated food waste, the initial hydrolysis appeared to plateau at 6 hours at which point secondary hydrolysis occurred from 6 to 24 h (Figure 3-23). The biphasic hydrolysis may have been due to the microbial nature of the inoculum. The first hydrolysis kinetic curve may have been a result of hydrolytic enzymes constitutively produced by the microorganisms and were present in the inoculum at the start of the assay. When the fresh food waste was added to the inoculum, the microorganisms must adapt to the new feedstock and induce further hydrolytic enzyme production. This adaptation period resulted in the second kinetic curve. To determine kinetic rates, initial (0-6 h) and secondary (6-24 h) hydrolysis were fit to individual first-order kinetic equations with the kinetic rate constant (k) and final hydrolyzed soluble fraction ($CODhsf_f$) as fitted parameters.

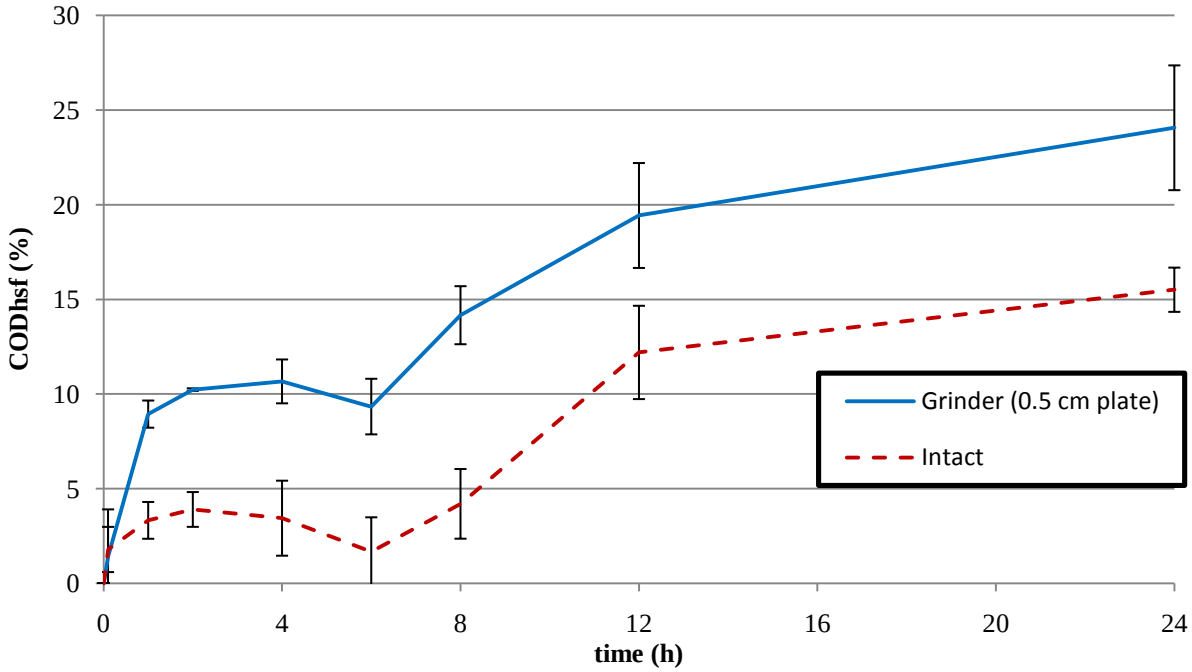


Figure 3-23. Mean hydrolysis in the microbial inoculum assay. Error bars represent standard deviation.

Estimates of parameters

Table 3-3 shows estimates of the fitted parameters and CODhsf at 6 h for the initial hydrolysis of pretreated and intact food waste. The rates of both food wastes were statistically similar, while pretreated food waste showed a significantly higher $CODhsf_f$.

Table 3-3. Estimated parameters and 6 h hydrolyzed COD for initial hydrolysis in the microbial inoculum assay (t=0 to 6 h)

	k (h^{-1})		$CODhsf_f$ (%)		$CODhsf_6$
Treatment	Estimate[a]	SE[b]	Estimate[a]	SE[b]	
Grinder (0.5 cm plate)	1.989 X	0.395	10.167 X	0.365	10.167
Intact	8.681 X	6.009	3.090 Y	0.376	3.087

a: Different letters in the same column indicate significant difference (α=0.05),
b: Standard error

Table 3-4 shows estimates of the fitted parameters and CODhsf at 24 h for the secondary hydrolysis of pretreated and intact food waste. The rates of both food wastes were statistically similar, while pretreated food waste showed a significantly higher $CODhsf_f$.

Table 3-4. Estimated parameters and 24 h hydrolysis for secondary hydrolysis in the microbial inoculum assay (t=6 to 24 h)

	k (h^{-1})		$CODhsf_f$ (%)		$CODhsf_{24}$
Treatment	Estimate[a]	SE[b]	Estimate[a]	SE[b]	
Grinder (0.5 cm plate)	0.184 X	0.037	25.418 X	1.179	24.861
Intact	0.168 X	0.048	17.854 Y	1.802	17.131

a: Different letters in the same column indicate significant difference (α=0.05)
b: Standard error

Fitted curves

Figure 3-24 shows the fitted curves for the initial and secondary hydrolysis with measured data points. Although the initial and secondary hydrolysis rates were similar, pretreated food waste showed significantly higher extents of hydrolysis over both hydrolysis periods. Within the first 2 h 10% of the TCOD of pretreated food waste was hydrolyzed, while only 3% of intact food waste was hydrolyzed. After 24 h, 25% of pretreated food was hydrolyzed, and 17% of intact food waste was hydrolyzed. The increased hydrolysis of pretreated food waste indicated that pretreatment increased the availability of the substrate to hydrolytic enzymes both in the initial hydrolysis and secondary hydrolysis.

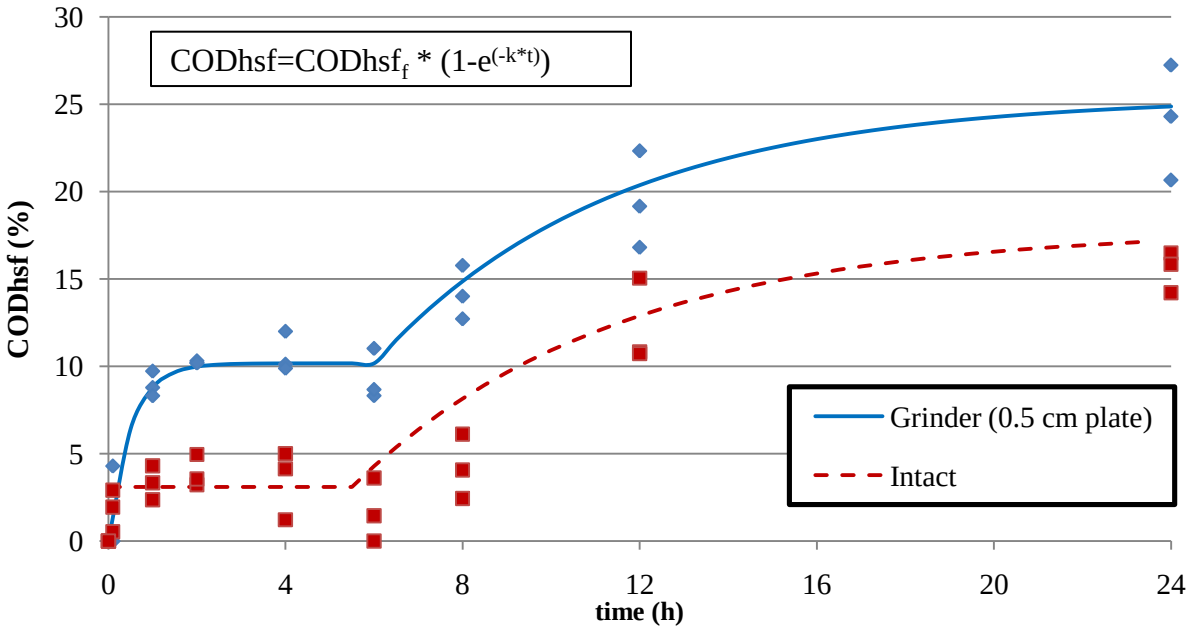


Figure 3-24. Fitted solubilization curves for the microbial inoculum assay

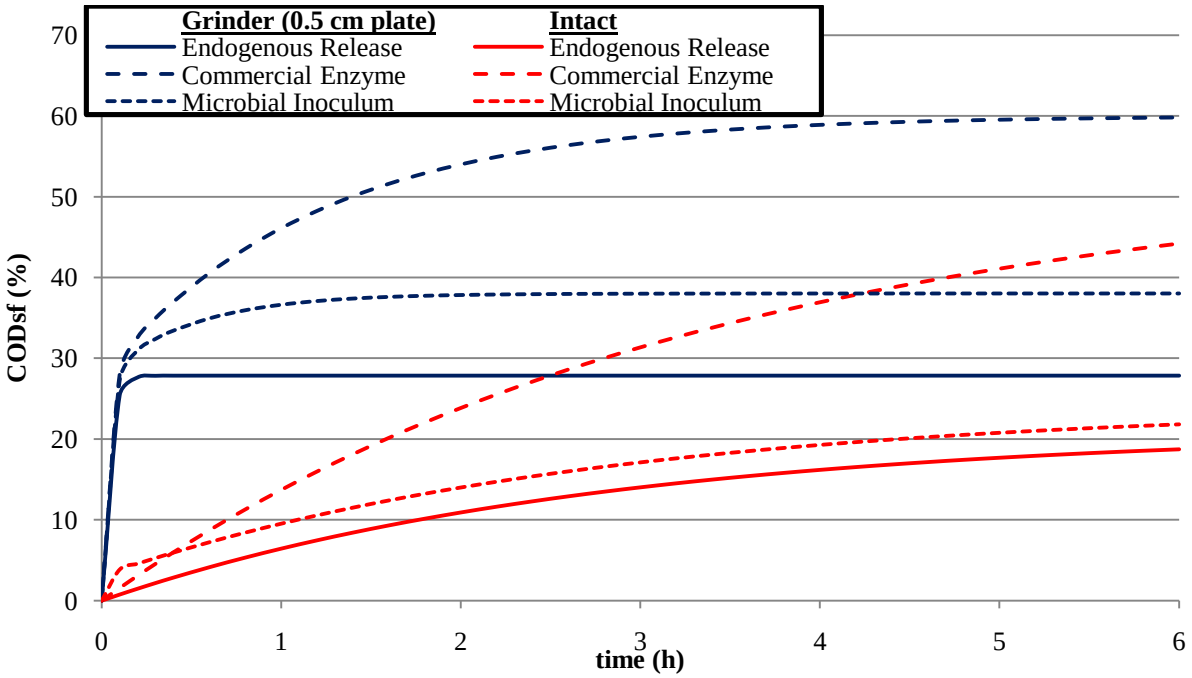


Figure 3-25. Six h solubilization of intact and pretreated (grinder 0.5 cm plate) food waste in the three solubilization assays.

Table 3-5. Maximum extent of solubilization for intact and pretreated (grinder 0.5 cm plate) food waste in the three solubilization assays

Assay	Food Waste	Maximum solubilization (CODsf)	Time at which maximized (h)	1st-order rate (k) (h^{-1})
Endogenous Solubilization	Pretreated	26.7 ± 0.65	< 1	24.3 [a]
Endogenous Solubilization	Intact	21.0 ± 0.22	8	0.36 [a]
Commercial Enzyme	Pretreated	60.9 ± 1.11	6	0.84 [b]
Commercial Enzyme	Intact	49.1 ± 5.52	8	0.254 [b]
Microbial Inoculum	Pretreated	51.9 ± 3.30	24	0.184 [c]
Microbial Inoculum	Intact	36.6 ± 1.17	24	0.17 [c]

a: Kinetic rate for release of endogenous SCOD
b: Kinetic rate for enzymatic hydrolysis
c: Kinetic rate for secondary enzymatic hydrolysis

Results from the solubilization assays indicated that mechanical pretreatment was very effective at increasing the solubilization kinetics of food waste. Figure 3-25 shows the combined solubilization curves for pretreated (grinder with 0.5 cm plate) and intact food waste in the first 6 h in all three assays. Table 3-5 shows the maximum extent of solubilization measured in the solubilization assays, time at which the solubilization maximized, and the estimated first-order

kinetic rates. Mechanical pretreatment caused immediate release of the endogenous soluble material within the food waste and increased food waste availability for hydrolytic enzymes. At 6 h, 28% of pretreated food waste is solubilized through the release of endogenous SCOD alone, while the addition of commercial enzymes increased solubilization to 60% (32% solubilization through enzymatic hydrolysis). Intact food waste was only 19% solubilized at 6 h through endogenous SCOD release, and this release did not occur immediately as with pretreated food waste. The addition of commercial enzymes to intact food waste increased solubilization to 44% (25% solubilization through enzymatic hydrolysis). Using a microbial inoculum, pretreated and intact food waste reached 52% and 37% solubilization by 24 h (Table 3-5). Using the natural microbial consortia present in an anaerobic digester, pretreated food waste is nearly as solubilized within 24 hours as it is within 6 h using excess commercial hydrolytic enzymes. Additionally the inoculum required an adaptation period due to the experimental conditions of the assay; this period would be negligible in an active, continuously-fed anaerobic digester. Therefore, with practical mechanical pretreatment, the hydrolytic enzymes produced by the microbial consortia are sufficiently effective to nullify any gain in solubilization by the addition of commercial hydrolytic enzymes.

A critical difference between intact and pretreated was the release of endogenous SCOD immediately following pretreatment (0.1 h in present study). Pretreatment immediately released the entire endogenous SCOD of the food waste (25% CODsf), while intact food waste was only 4% solubilized (Figure 3-10). The initial solubilization of pretreated food waste in the present study was comparable to literature values using more intensive pretreatment methods. Table 3-6 shows the values for the initial food waste solubilization calculated from literature studies. It is important to note that all the literature studies used pretreated food waste (i.e. shredded or

ground) as a control and that a significant amount of endogenous SCOD had already been released prior to the pretreatment used in the studies. Izumi et al. (2010), which found an immediate solubilization of 28% using a food disposer, increased initial solubilization to 39%, with 300 revolutions (1 minute at 300 rpm) of a ball mill. However, using a ball at 40,000 revolutions (20 minutes at 2000 rpm), initial solubilization was still only 40% solubilized. This showed that minimal ball milling released the full complement of endogenous SCOD, and the majority of which was released through the control pretreatment using a food disposer. Other studies showed similar initial solubilization of pretreated food waste, 17% to 32% through thermal and freezing/thawing pretreatment, while control (shredded) food waste was 8% to 17% solubilized (Liu et al., 2008; Wang et al., 2006). However, some of this increase may have been due to thermal hydrolysis when heating food waste in addition to released endogenous SCOD. Different types of food wastes have different endogenous SCOD; therefore, it is important to consider the increase of pretreated food waste over controls. Because the literature values were using shredded or ground food waste as a control, the gain in initial solubilization through additional pretreatment is much less than the gain achieved over intact food waste. The additional pretreatment using energy-intensive methods only showed marginal gains in endogenous SCOD release compared to the large gain using practical pretreatment (shredding, disposer, or grinder). Also, the endogenous SCOD of the food waste in the present study was measured at 25%, all of which is released immediately through pretreatment with the grinder (0.5 cm plate). Therefore, additional intensive pretreatment would not release additional endogenous SCOD.

The present study showed that pretreatment increased food waste's availability to hydrolytic enzymes when incubated with a microbial inoculum for 24 h. Pretreated and intact

food waste was 52% and 37%, respectively, solubilized within 24 h. These values were within the range found in literature that measured solubilization of pretreated food waste in acidogenic reactors. Table 3-7 shows the calculated literature values for 24 h solubilization in an acidogenic reactor. Wang et al. (2006) thermally pretreated food waste by heating to 70°C for 2 h or 150°C for 1 h. In 24 h, control food waste (shredded), 70°C pretreatment, and 150°C pretreatment were 31%, 37%, and 57% solubilized, respectively. Liu et al. (2008) also examined thermally pretreated food waste (150°C for 1 h), as well as frozen/thawed food waste. Control and thermally pretreated food waste were 17% and 31%, respectively, solubilized within 24 h, and control and frozen/thawed food waste were, 24% and 33%, respectively solubilized by 24h. Kim et al. (2005) used a hydrolytic enzyme cocktail, at a rate of 0.2% (v/v), for food waste pretreatment in an acidogenic reactor. Control and pretreated food waste showed a 24 h CODsf of 37.5% and 52.5%, respectively. However, the present study found a similar 24 h solubilization (51.9%) without the addition of commercial enzymes using practical pretreatment and the microbial consortia present in an anaerobic digester. The values in the present studies, while similar to literature values, were achieved without the use of expensive or energy-intensive pretreatments. Therefore, it can be concluded that practical mechanical pretreatment methods are as effective as the intensive pretreatment methods in literature at increasing the release of endogenous soluble organic material and the enzymatic hydrolysis of food waste.

Table 3-6. Initial solubilization of food waste in pretreatment studies

Source of food waste	Control treatment	Pretreatment method	Control CODsf (%)	Pretreated CODsf (%)	Increase in CODsf (%)[a]	Source
University dining	shredded	2 h @ 70 ^{o}C	17.2	20.3	3.1	Wang et al. 2006
University dining	shredded	1 h @ 150 ^{o}C	17.2	31.7	14.5	Wang et al. 2006
University dining	shredded	1 h @ 150 ^{o}C	7.8	16.9	9.1	Liu et al. 2008
University dining	shredded	frozen 24 hr @ - 20 ^{o}C	14.9	25.4	10.5	Liu et al. 2008
Standard food waste	food disposer	ball mill (300 revolutions)	28.1	39.0	10.9	Izumi et al.2010
Standard food waste	food disposer	ball mill (40,000 revolutions)	28.1	40.3	12.3	Izumi et al.2010
Standard food waste	intact	meat grinder 0.5 cm plate	4.3	25.3	21.0	Current study
Standard food waste	intact	meat grinder 1.0 cm plate	4.3	19.6	15.3	Current study
Standard food waste	intact	food disposer	4.3	21.1	16.8	Current study

a: Increase is difference between pretreated CODsf and control CODsf
Note: Values are calculated from the respective literature

Table 3-7. One-day solubilization of food waste in pretreatment studies.

Source of food waste	Control treatment	Pretreatment method	Loading rate (g COD/L)[a]	Solubilization temperature	1 d CODsf (%) Control	1 d CODsf (%) Pretreated	Increase in CODsf (%)[b]	Source
University dining	shredded	2 h @ 70°C	42.1	35°C	30.9	36.9	6.0	Wang et al. 2006
University dining	shredded	1 h @ 150°C	42.1	35°C	30.9	57.1	26.2	Wang et al. 2006
University dining	shredded	1 h @ 150°C	45.1	35°C	16.6	31.1	7.2	Liu et al. 2008
University dining	shredded	frozen 24 h @ -20°C	54.3	35°C	23.9	33.1	9.2	Liu et al. 2008
University dining	blended	enzyme cocktail	20.0	35°C	37.5	52.5	15.0	Kim et al. 2005
Standard food waste	intact	meat grinder 0.5 cm plate	3.5	35°C	36.6	51.9	15.3	Current study

a: Loading rates in literature studies are for the acidogenic reactor in a two-phase system
b: Increase is difference between pretreated CODsf and control CODsf
Note: Values are calculated from the respective literature

Summary and Conclusions

Three solubilization assays were conducted to compare the solubilization kinetics of intact food waste to mechanically pretreated food waste. The three assays measured 1) the release of endogenous SCOD, 2) enzymatic hydrolysis using commercial enzymes and 3) a microbial inoculum. Pretreatment of food waste using an in-sink food disposer and meat grinder (with either 1.0 or 0.5 cm plate openings) resulted in significantly higher solubilization kinetics than intact food waste. The release of endogenous SCOD occurred immediately in pretreated food waste, while endogenous SCOD from intact food waste was released at a rate of 0.36 h^{-1}. Intact food waste was only 4.1% solubilized initially, while pretreated food waste was 19% to 25% immediately following pretreatment. The application of commercial enzymes allowed the enzymatic hydrolysis of food waste to be measured. Within 8 h, 61% and 49%, respectively, of intact and pretreated food waste were solubilized, which corresponded to 30 to 32% enzymatic hydrolysis for both food wastes. Although the extent of enzymatic hydrolysis between intact and pretreated food waste were similar within 8 h, the rates of hydrolysis were significantly higher for pretreated food waste (0.8-1.2 h^{-1}) than intact food waste (0.3 h^{-1}). This indicated an increase in the immediate availability of the pretreated food waste to hydrolytic enzymes due to looser physical structure and reduced particle size. A microbial inoculum also showed increased solubilization of pretreated food waste over intact food waste. Within 24 h of incubation with the microbial inoculum, 52% of pretreated food waste and 37% of intact food waste was solubilized. Pretreated food waste was nearly as solubilized in 24 h with a microbial inoculum, as in 8 h with excess commercial hydrolytic enzymes. In an active, continuously-fed anaerobic digester, the microbial hydrolysis would show more rapid kinetics due to the lack of the adaptation period that was present in the assay. This indicated that the hydrolytic enzymes produced by microbial consortia could sufficiently solubilize pretreated food waste without the

addition of expensive, commercial enzymes. The three solubilization assays showed that the use of practical mechanical pretreatment methods, through disrupting tissue and rupturing cells, greatly enhanced the short-term solubilization kinetics of food waste.

CHAPTER 4
EFFECT OF MECHANICAL PRETREATMENT ON BIOMETHANATION OF FOOD WASTE

Anaerobic digestion is a sequential metabolism that is performed by mixed microbial consortia. Particulate feedstocks, such as food waste, must first be solubilized through enzymatic hydrolysis. The soluble organic material is then fermented into organic acids, which are ultimately metabolized into acetic acid. Methanogens consume acetic acid and produce methane as a metabolic byproduct. For the anaerobic digestion of high particulate feedstocks the rate-limiting step is considered to be hydrolysis (Eastman & Ferguson, 1981; Izumi et al., 2010; Palmowski & Muller, 2003; Wang et al., 2006). As the first-step in the process, rate-limitation by hydrolysis can hinder the entire digestion process, which leads to reduced biogas production and an increased reactor size needed to treat a given volume of waste. Therefore, increasing the rate of hydrolysis and solubilization through pretreatment of food waste is proposed as a method to increase the overall efficiency of food waste digestion. Literature studies have indicated that various pretreatment methods enhance the solubilization and subsequent methane production kinetics of food waste (Izumi et al., 2010; Liu et al., 2008; Wang et al., 2006). The pretreatment methods reported in literature, however, are energy and resource intensive and are not economically feasible for widespread implementation of food waste anaerobic digestion. The present study assessed the enhancement of anaerobic digestion using low-tech, practical pretreatment methods. Pretreating food waste with a manual meat grinder and in-sink disposer was shown to significantly increase food waste solubilization compared with intact food waste. Therefore, it was hypothesized that increased solubilization of pretreated food waste provides increased substrate available for methanogenesis, which can potentially increase biomethanation kinetics. To test this hypothesis, biochemical methane potential (BMP) assays were conducted on pretreated and intact food waste.

The BMP assay is used as a laboratory-scale method for measuring the methane production kinetics of a feedstock under batch loading conditions (Owen et al., 1979). The assay utilizes a mixed microbial inoculum from a methanogenic source, which contains the required consortia of microorganisms necessary for hydrolysis, acidogenesis, acetogenesis and methanogenesis to occur. As the microbial consortia digest the feedstock, methane production is measured over time, which is used to calculate the degradation rate of the feedstock. This measurement allows both the rate and extent of feedstock degradation to be determined under simulated anaerobic digester conditions. A loading rate of 2 to 4 g COD/L is typically used in the BMP assay because at higher loading rates, acidification can occur which lowers the pH in the assay and inhibits methanogenesis. Because the BMP assay is a batch digestion process, proper inoculum selection is critical for optimum results. The assay is designed with excess nutrients, alkalinity, and inoculum so that under proper loading rates the only limiting factor is the degradation rate of the feedstock itself. For this reason, the BMP assay was utilized in the present study to compare the methane production kinetics of intact food waste to pretreated food waste.

Moderate-Loading-Rate Biochemical Methane Potential Assay

Results from three solubilization assay indicated that pretreated food waste had significantly higher solubilization kinetics than intact food waste (Chapter 3). Therefore, a BMP assay was conducted at the same loading rate as the solubilization assays, 10 g (ww)/L, which corresponded to a nominal COD loading rate of 3.5 g COD/L, a moderate loading rate for the typical range of the BMP assay. In the BMP assay, pretreated food waste was represented by the meat grinder (0.5 cm plate) pretreatment method. In the solubilization assays (Chapter 3), all three practical pretreatment methods showed approximately similar increases in food waste solubilization. The meat grinder with 0.5 cm plate openings was selected as the pretreatment method for the BMP assay because this method produced more homogenous pretreated food

waste for obtaining representative samples, which is critical for the BMP assay due to the small mass of substrate used (Wilkie et al., 2004). Flushed dairy manure (100% v/v) was used as an inoculum for the assay, because it contains the mixed microbial consortia and sufficient nutrients and alkalinity needed for anaerobic digestion (Wilkie, 2005). The assay included a glucose and cellulose control loaded at 2 g/L (2.13 g COD/L) to measure the activity of the inoculum. An inoculum blank was also measured in the assay to account for any methane production from the COD of the inoculum.

Cumulative Methane Production

Cumulative methane production was measured for 30 d in the assay. Methane production measurements were normalized to per g COD loaded and STP conditions after subtracting methane production from the inoculum blank. Cumulative methane production was modeled to a first-order kinetic equation with the kinetic rate constant (k) and ultimate cumulative methane yield (CH_{4f}) as fitted parameters.

Figures 4-1 and 4-2 show the fitted model against measured data points for food waste and controls, respectively. Table 4-1 shows estimates of fitted parameters for the kinetic models for intact and pretreated food waste and glucose and cellulose controls. Intact food waste showed a significantly higher rate (k) and extent (CH_{4f}) of methane production than pretreated food waste at α=0.05. However at α=0.01 methane production rates were similar between each food waste. Table 4-2 shows the percent COD removal at 5, 10, 20, and 30 d based on the stoichiometric COD equivalent of methane (2.86 g COD/L CH_4 @ STP). The assays showed that by 20 d, over 90% of intact food waste was converted to methane, while 81% of pretreated waste was converted at 20 d, and by 30 days, only 84% of pretreated food waste had been converted. Table 4-3 shows the total COD, soluble COD, pH and conductivity measured on the digestate at the

end of the assay. After 30 days, there was more unconverted TCOD from pretreated food waste than intact food waste, as there was less methane produced from the pretreated food waste.

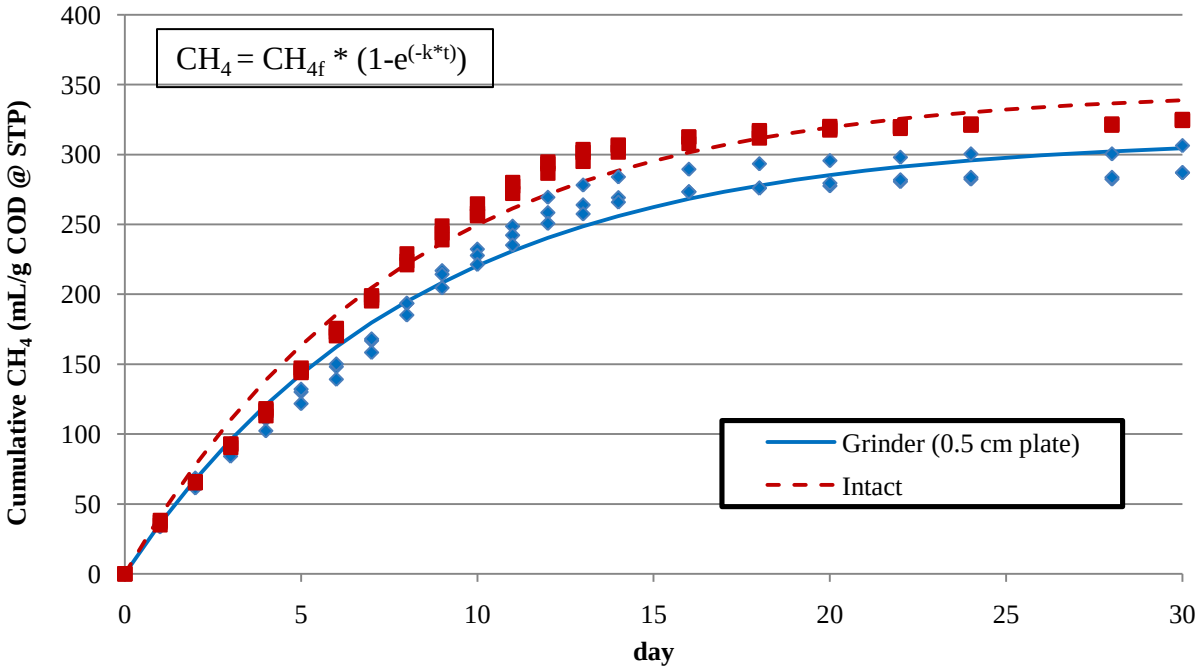


Figure 4-1. Cumulative methane production of intact and pretreated food waste in the moderate-loading-rate BMP assay. Pretreatment is meat grinder (0.5 cm plate).

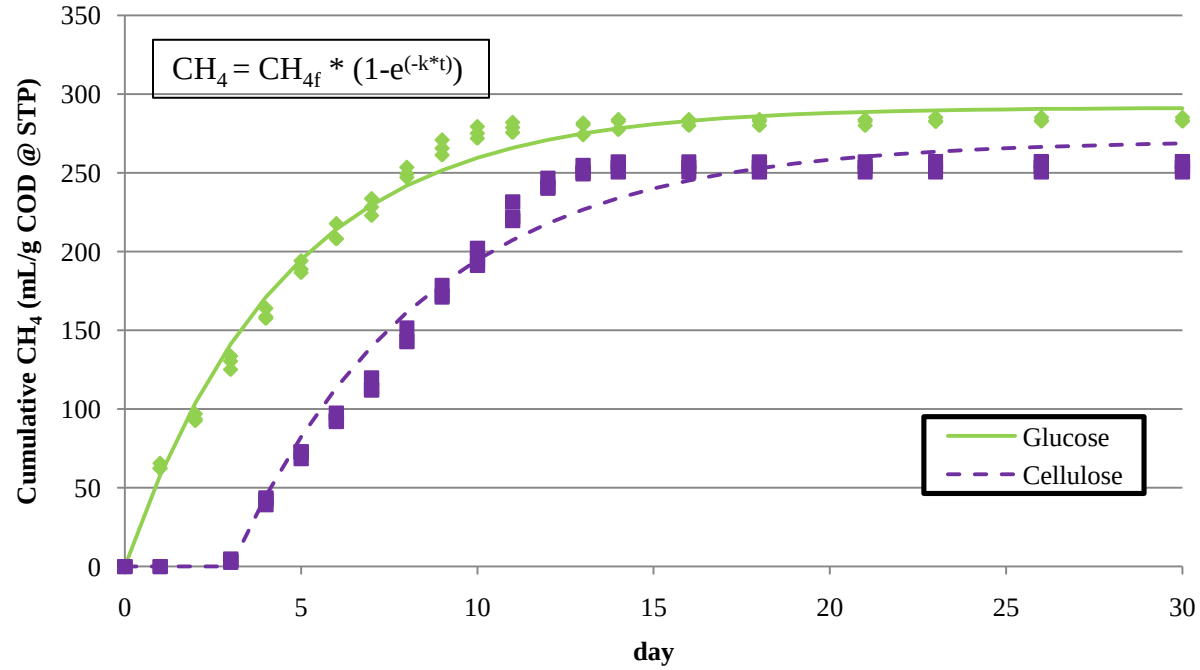


Figure 4-2. Cumulative methane production from glucose and cellulose controls in the moderate-loading-rate BMP assay.

Table 4-1. Estimates of parameters for methane production kinetics in the moderate-loading-rate BMP assay.

	k (day^{-1})		CH_{4f} (mL/g COD added)	
	Mean[a]	Standard error	Mean[a]	Standard error
Pretreated	0.122 W	0.0022	312.32 W	2.23
Intact	0.128 X	0.0022	346.25 X	2.28
Glucose control	0.221 Y	0.0036	291.44 Y	1.40
Cellulose control	0.182 Z	0.0002	270.60 Z	0.25

a: Different letters in the same column indicate significant difference (α=0.05)

Table 4-2. Calculated COD removal at 5, 10, 20 and 30 days in the moderate-loading-rate BMP assay.

	% COD removal[a]			
	Day 5	Day 10	Day 20	Day 30
Pretreated	36.6 ± 1.57	64.9 ± 1.57	81.2 ± 2.83	83.8 ± 3.19
Intact	41.6 ± 0.47	74.2 ± 1.28	91.1 ± 0.38	92.8 ± 0.11
Glucose control	54.2 ± 1.11	78.7 ± 1.08	80.6 ± 0.56	81.1 ± 0.37
Cellulose control	20.4 ± 0.71	56.0 ± 1.57	72.5 ± 0.92	72.5 ± 0.96

a: based on COD equivalent of cumulative methane production
Note: Mean values ± one standard deviation

Table 4-3. Total COD, SCOD, pH, and conductivity after 30 days of digestion in the moderate-loading-rate BMP assay.

	TCOD (g/L)	SCOD (g/L)	pH	Conductivity (mS/cm)
Pretreated	2.64 ± 0.09	0.488 ± 0.005	6.96 ± 0.01	3.50 ± 0.10
Intact	2.40 ± 0.06	0.482 ± 0.004	6.94 ± 0.01	2.93 ± 0.27
Glucose control	2.65 ± 0.05	0.488 ± 0.006	6.86 ± 0.01	3.14 ± 0.04
Cellulose control	2.93 ± 0.17	0.596 ± 0.015	6.84 ± 0.02	3.15 ± 0.32
Inoculum blank	2.29 ± 0.05	0.379 ± 0.009	7.04 ± 0.02	3.00 ± 0.03

Note: Mean values ± one standard deviation

The results of this assay indicated that, at a loading rate of 3.5 g COD/L, pretreated food waste did not have higher methanogenic kinetics than intact food waste. It is likely that the loading rate exceeded the capacity of the BMP assay. Exceeding the loading rate would result in increased acidogenesis, which would lower the pH and inhibit methanogenesis. The high solubilization of pretreated food waste can exacerbate the problem due to more rapid

acidogenesis than intact food waste. Despite a neutral pH by 30 d (Table 4-3), it is likely that a period of reduced pH at inhibitory levels occurred in the assay for pretreated food waste.

Loading Rate and pH

Results from the BMP assay indicated that a moderate loading rate of 3.5 g COD/L may have caused inhibited biomethanation of pretreated food waste due to acidification. Therefore for further study of food waste biomethanation, it was critical to determine the effect of loading rate on pH. To assess this effect, a simulated BMP assay was conducted at loading rates of 2, 4 and 8 g COD/L for intact and pretreated food waste. To measure pH, 10 mL subsamples were taken from each bottle over 20 d.

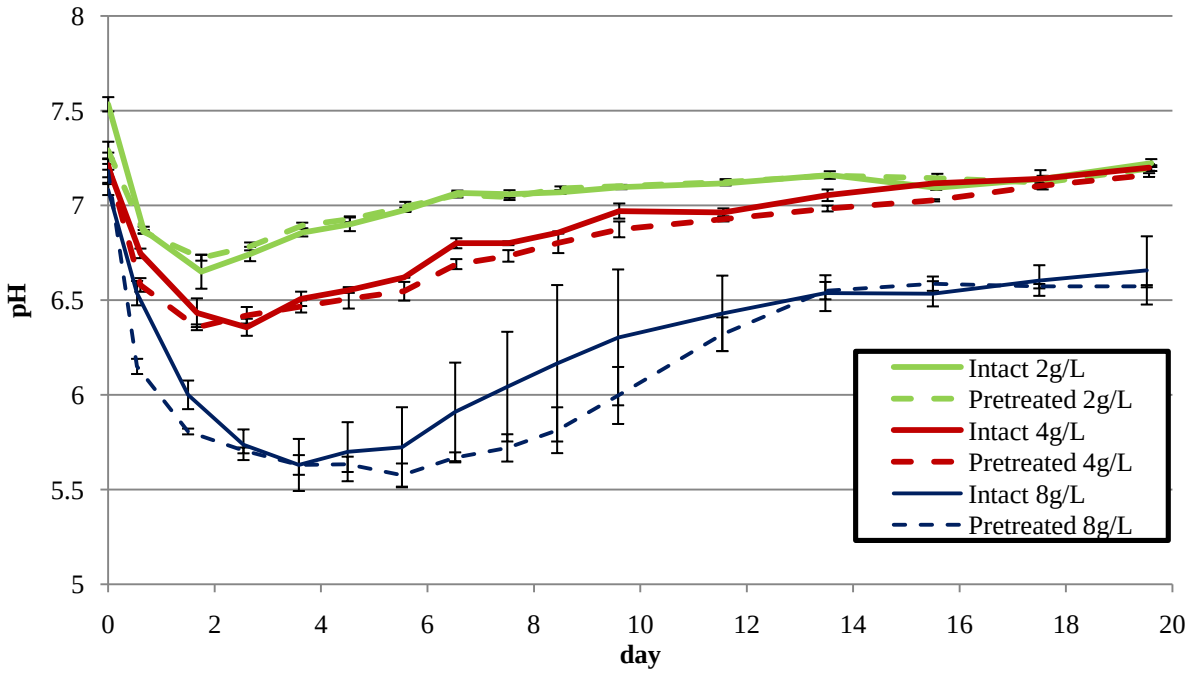


Figure 4-3. Mean pH in simulated BMP assays loaded at 2, 4, and 8 g COD/L with intact and pretreated food waste. Error bars represent standard deviation.

Figure 4-3 shows the mean pH for intact and pretreated food waste at each loading rate. Within 2 days at a loading rate of 4 g COD/L pH dropped below 6.5, which is considered the minimum optimum pH for methanogenesis (Speece, 2008), for both intact and pretreated food waste. Pretreated food waste, however, showed a faster pH decrease and remained below the pH

of intact food waste for most of the 20 d period, which suggested that the enhanced solubilization of pretreated food waste resulted in increased acidogenesis. Increased acidogenesis can inhibit methanogenesis if the methanogens cannot consume the increased organic acids and the acids accumulate. This is clearly exacerbated at a loading rate of 8 g COD/L, which is far above the capacity of the BMP assay, where pH dropped to nearly 5.5 and recovered to only 6.5 within 20 d. At a loading rate of 2 g COD/L, pH did not drop below 6.5 and intact and pretreated food waste followed approximately the same pH drop and recovery. Therefore, acidification would not become inhibitory for methanogenesis at this reduced loading rate.

Reduced-Loading-Rate Biochemical Methane Potential Assay

To lessen the impact of acidification on methanogenesis in the BMP assay, the loading rate was reduced to 2 g COD/L. The assay compared pretreated (grinder 0.5 cm plate) against intact food waste with glucose and cellulose controls. The inoculum used in this assay was the conserved digestate from the moderate-loading-rate BMP. The conserved inoculum had lower total and soluble COD compared with fresh flushed dairy manure, which further reduced the potential for acidification through COD overloading.

Cumulative Methane Production

Methane production was measured for 30 days in the assay. All methane production measurements were normalized to per g COD loaded and STP conditions after subtracting methane production from the inoculum blank. Cumulative methane production was modeled to a first-order kinetic equation with the kinetic rate constant (k) and ultimate cumulative methane yield (CH_{4f}) as fitted parameters.

Figures 4-4 and 4-5, show the fitted model against measured data points for food waste and controls, respectively. Table 4-4 shows estimates of fitted parameters for the kinetic models for intact and pretreated food waste and glucose and cellulose controls. With the reduced loading

rate, pretreated food waste had a significantly higher kinetic rates (0.21 d^{-1}) than intact food waste (0.20 d^{-1}) at α=0.05, although the rates were statistically similar at α=0.01. Pretreated food waste also showed a statistically higher COD; however, this may be an effect of the model because the measured cumulative methane yields at 30 d are the same for both food wastes (Figure 4-5). Tables 4-5 shows the percent COD removal at 5, 10, 20, and 30 d based on the stoichiometric COD equivalent of methane (2.86 g COD/L CH_4 @ STP). Both food wastes are approximately 60% converted to methane by 5 d and approximately 90% converted by 20 d. Table 4-6 shows the total COD, soluble COD, pH, conductivity, and alkalinity measured on the digestate at the end of the assay. After 30 days of digestion, digestate from intact and pretreated food waste had similar TCOD, SCOD, pH, and alkalinity. Therefore at a loading rate of 2 g COD/L, both intact and pretreated food waste had similar methane production kinetics under the conditions of the BMP assay.

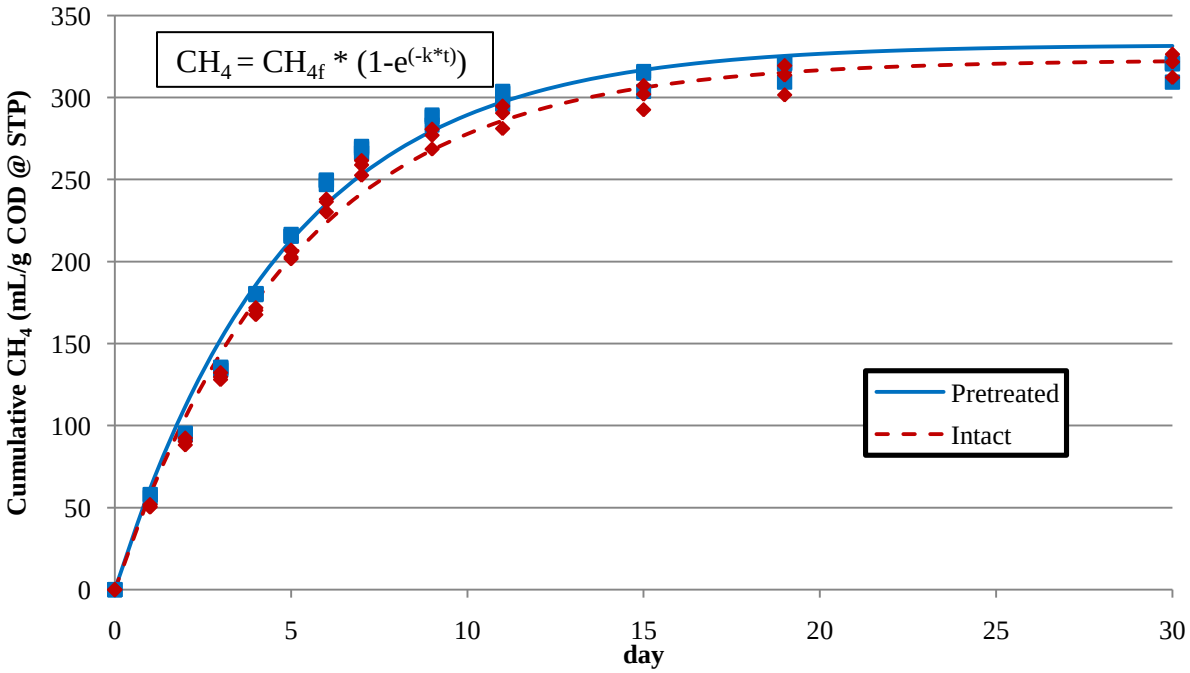


Figure 4-4. Cumulative methane production of intact and pretreated food waste in the reduced-loading-rate BMP assay. Pretreatment is meat grinder (0.5 cm plate).

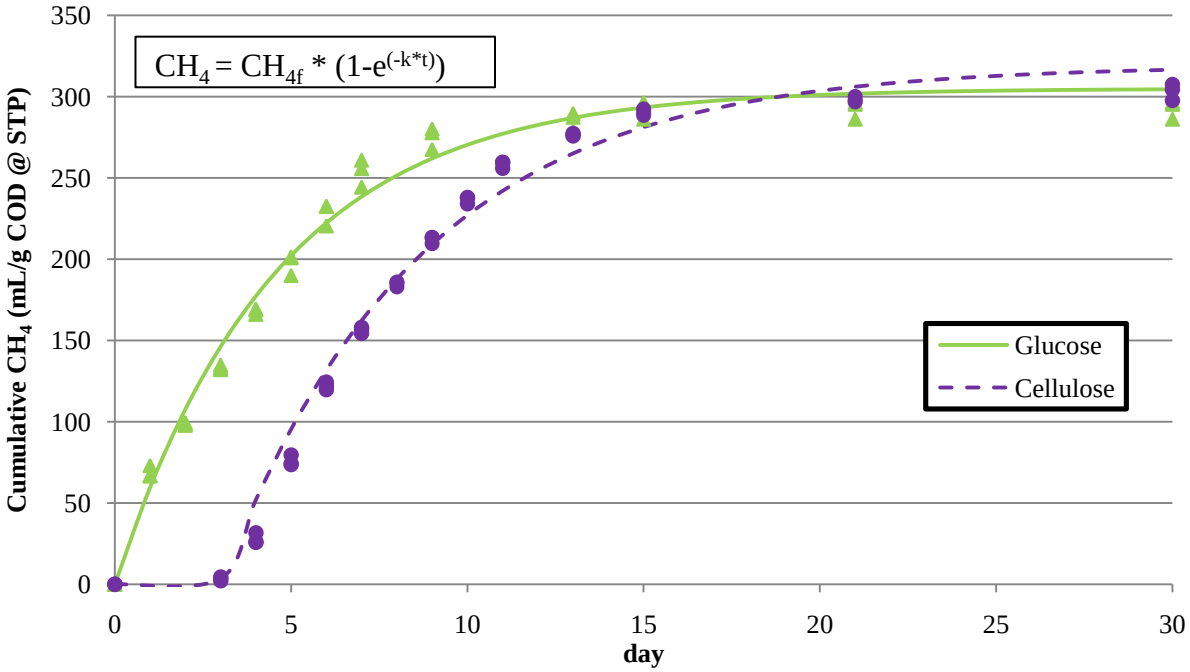


Figure 4-5. Cumulative methane production of glucose and cellulose controls in the reduced-loading-rate BMP assay.

Table 4-4. Estimates of parameters for methane production kinetics in the reduced-loading-rate BMP assay.

	k (day^{-1})		CH_{4f} (mL/g COD added)	
	Mean[a]	Standard error	Mean[a]	Standard error
Pretreated	0.205 W	0.0039	332.119 X	2.53
Intact	0.197 X	0.0037	323.028 Y	2.46
Glucose control	0.218 Y	0.0044	304.899 Z	2.31
Cellulose control	0.177 Z	0.0040	319.076 Y	3.05

a: Different letters in the same column indicate significant difference (α=0.05)

Table 4-5. Calculated COD removal at 5, 10, 20 and 30 days in the reduced-loading-rate BMP assay.

	COD removal (%)[a]			
	Day 5	Day 10	Day 20	Day 30
Pretreated	61.7 ± 0.18	84.0 ± 1.07	90.6 ± 1.89	90.6 ± 1.89
Intact	58.2 ± 0.79	80.6 ± 1.88	89.4 ± 2.60	91.4 ± 2.06
Glucose control	56.4 ± 1.84	81.0 ±1.78	83.6 ± 1.61	83.6 ± 1.61
Cellulose control	21.7 ± 0.95	67.6 ± 0.62	84.4 ± 0.48	86.6 ± 1.45

a: based on COD equivalent of cumulative methane production
Note: Mean values ± one standard deviation

Table 4-6. Total COD, soluble COD, pH, conductivity, and alkalinity after 30 days of digestion in the reduced-loading-rate BMP assay.

	Total COD (g/L)	Soluble COD (g/L)	pH	Conductivity (mS/cm)	Alkalinity (mg $CaCO_3$ eq./L)
Pretreated	2.63 ± 0.06	0.388 ± 0.017	6.97 ± 0.02	3.81 ± 0.09	2600 ± 25.0
Intact	2.56 ± 0.06	0.385 ± 0.002	6.96 ± 0.01	3.65 ± 0.04	2568 ± 12.0
Glucose control	2.63 ± 0.04	0.393 ± 0.012	6.90 ± 0.01	3.37 ± 0.02	2344 ± 13.9
Cellulose control	2.80 ± 0.15	0.513 ± 0.013	6.88 ± 0.01	3.50 ± 0.04	2312 ± 79.9
Inoculum blank	2.33 ± 0.09	0.370 ± 0.024	7.22 ± 0.01	3.37 ± 0.02	2384 ± 6.9

Note: Mean values ± one standard deviation

Discussion

Compared to the moderate-loading-rate BMP assay, the methane production kinetics of both intact and pretreated food waste were greater at the reduced-loading rate. Figure 4-6 compares the cumulative methane production of food waste in the two assays. Over the first 10 d, methane production was greater at the reduced loading than in the moderate-loading-rate assay. The decreased methane production correlated to decreased pH at a moderate loading rate (Figure 4-3). In the simulated BMP assay, pH remained below 7 for the first 10 d at a loading rate of 4 g COD/L. After 10 d, acidification from the intact food was overcome and cumulative methane production was approximately equal to that of intact and pretreated food waste loaded at 2 g COD/L. However, methane production and pH of pretreated food waste remained below that of intact food waste after 10 days at the moderate loading rate. These two assays demonstrated the importance of maintaining proper pH in an anaerobic digester, especially within the initial stages of digestion. In fact, in a low-rate digester, such as in the BMP assay, the pH appeared to be more critical to efficient anaerobic digestion than increasing solubilization through pretreatment. At a high loading rate, pretreatment can reduce methane production, while at a low loading rate, pretreatment does not appear to increase methane kinetics. Therefore, to increase the efficiency of methane production from pretreated food waste, it is necessary to utilize high-rate anaerobic digestion, such as a fixed-film reactor, to accommodate the higher rate of

solubilization and acidogenesis by facilitating a greater metabolic flux of the acidogenic products. A high-rate digester can thus maintain neutral pH conditions due to more rapid consumption of organic acids by the methanogenic consortia.

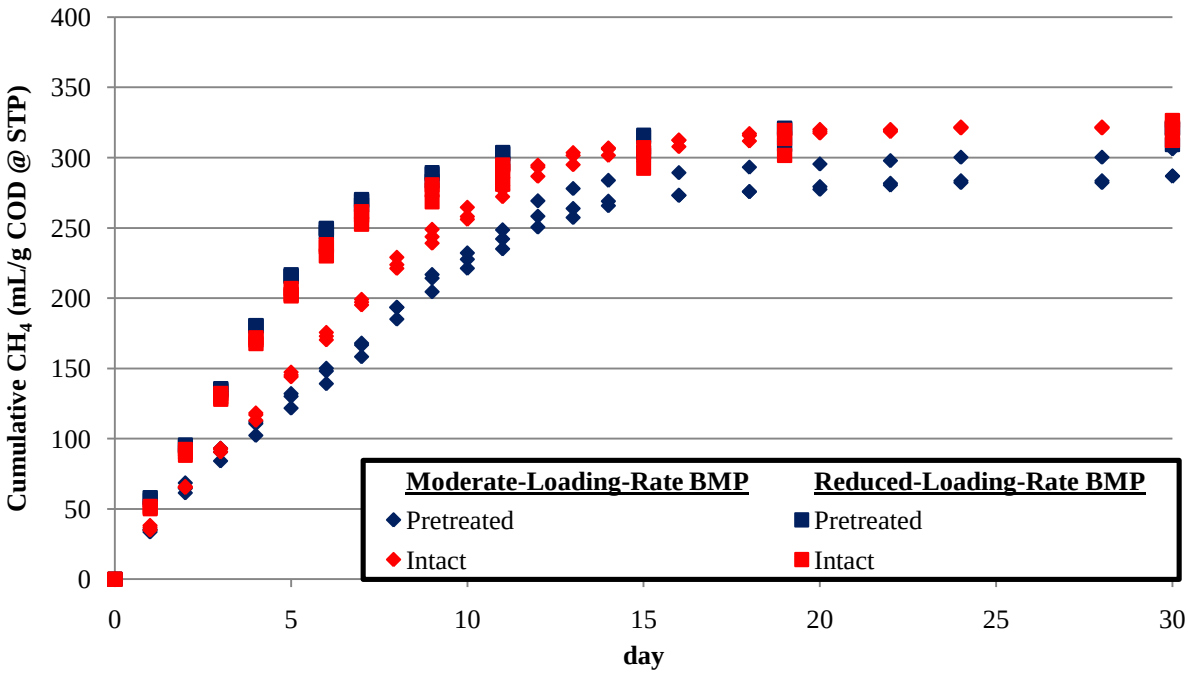


Figure 4-6. Cumulative methane production of pretreated and intact food waste in both BMP assays.

The moderate-loading-rate and reduced-loading-rate BMP assays in this study indicated that food waste is a readily degradable substrate for anaerobic digestion. At a proper loading rate (2 g COD/L for the BMP assay), food waste is more than 80% converted to methane within 10 d and 90% converted within 20 d (Table 4-5). Table 4-7 lists literature values for 10 d cumulative methane yield in studies examining food waste pretreatment. The present study found the highest 10 d methane yields for control food waste, at 282 mL/g COD and the second highest for pretreated food waste, at 294 mL/g COD. Because the 10 d methane yield of intact food waste is so great, the BMP assay is not able to show an increase for pretreated food waste. The methanogenic rate of the BMP assay cannot accommodate the increased solubilization of pretreated food waste.

The methane yield from the control is particularly interesting because the intact control in the present study showed greater methanogenesis than pretreated (shredded/ground) controls in the literature studies. Izumi et al. (2010) used a loading rate of 10 g COD/L and produced a 10 d methane production of 234 mL/g COD for the control food waste. The lower methane production than the present study may be an effect of the food waste or inoculum used in the experiment, as the pH remained neutral throughout the 10 d even at a loading rate of 10 g COD/L. The authors were able to increase 10 d methane production to 320 mL/g COD using a ball mill pretreatment for 1000 revolutions (2 minutes at 500 rpm). However, with further ball milling for 40,000 revolutions (20 minutes at 2000 rpm), methane production was reduced to 252 mL/g COD, which was concluded to be caused by acidification and inhibitory VFA levels due to excessive pretreatment and solubilization. These results support the findings in the present study that pretreatment of food waste can cause acidification through excessive solubilization at high loading rates.

To overcome the dilemma of acidification through pretreatment, an anaerobic digester design that can handle high concentrations of organic acids is necessary. Wang et al (2006) and Liu et al. (2008) examined the biomethanation kinetics of pretreated food waste using a hybrid anaerobic solid-liquid (HASL) system. The HASL system is a two-phase digester that employs an acidogenic reactor and a modified upflow anaerobic sludge blanket as a methanogenic reactor (Liu et al., 2010). It was designed for more optimum digestion of food waste. The ability to handle a higher loading rate, allows the HASL system to show improved biomethanation kinetics of pretreated food waste. Using the HASL system, Wang et al (2006) increaed 10 d methane yields by 16 and 40 mL/g COD for thermally pretreated food waste over control, and Liu et al. (2008) increased 10 d methane yields by 48 and 33 mL/g COD, respectively, for thermally

pretreated and frozen/thawed food waste over control. These studies suggest that by using a high-rate anaerobic digester, such as the HASL or a fixed-film reactor, pretreatment of food waste can increase biomethanation kinetics.

Table 4-7. Ten day cumulative methane production of food waste in pretreatment studies.

Food waste source	Control treatment	Pretreatment method	Loading rate (g COD/L)	Digestion temperature	10 day cumulative CH_4 (mL/g COD)		Increase[b] (mL/g COD)	Source
					Control	Pretreated		
University dining	shredded	2 h @ 70°C	<10 [a]	35°C	198.1	214.0	15.9	Wang et al. 2006
University dining	shredded	1 h @ 150°C	<10 [a]	35°C	198.1	237.8	39.7	Wang et al. 2006
University dining	shredded	1 h @ 150°C	<10 [a]	35°C	206.0	253.5	47.5	Liu et al. 2008
University dining	shredded	frozen 24 h @ -20°C	<10 [a]	35°C	197.3	230.2	32.9	Liu et al. 2008
Standard food waste	food disposer	ball mill (1000 revolutions)	10	37°C	234.2	320.0	85.8	Izumi et al.2010
Standard food waste	food disposer	ball mill (40,000 revolutions)	10	37°C	234.2	251.6	17.4	Izumi et al.2010
Standard food waste	intact	grinder 0.5 cm plate	2	35°C	282.1	293.9	11.8	Current study

a: The digester used was a two-phase system. Effluent from the methanogenic reactor was used to dilute the leachate from the acidogenic reactor to maintain proper organic loading rate.

b: Increase is difference between pretreated cumulative CH_4 and control cumulative CH_4.

Note: Values are calculated from the respective literature.

Summary and Conclusions

Two biochemical methane production assays were performed on intact and pretreated (grinder with 0.5 cm plate) food waste. The first assay was loaded at a moderate loading rate (3.5 g COD/L) using flushed dairy manure as an inoculum, while the second assay was loaded at a reduced loading rate (2 g COD/L) using the conserved digestate from the initial assay as an inoculum. The extent of degradation and first-order rate constants for the assays were estimated by non-linear fitting of parameter estimates on triplicate data sets. In both assays pretreated and intact food waste showed approximately similar first-order rate constants: 0.12 d^{-1} at 3.5 g COD/L and 0.20 d^{-1} at 2 g COD/L. At a loading rate of 3.5 g COD/L, intact food waste showed a greater 30 d methane yields (325 mL/g COD) than pretreated food waste (294 mL/g COD). While at 2 g COD/L, both intact and pretreated showed approximately similar 30 d methane yields (320 g COD/L). The decreased kinetics at the higher loading rate were attributed to acidification through increased acidogenesis. This was confirmed by measuring pH in a simulated BMP assay, where intact food waste at 4 g/L decreased in pH to below 6.5 in the first 3 d. Pretreated food waste showed a more depressed pH at the moderate loading rate, which suggests that pretreatment at excessive loading rates can inhibit methanogenesis through acidification.

While the reduced-loading-rate (2 g COD/L) BMP assay showed increased methane production over the moderate-loading-rate (3.5 g COD/L) assay, pretreated food waste did not show increased methane production over intact food waste. Therefore, it can be concluded that under the conditions of the BMP assay, pretreatment of food waste does not enhance the rate of biomethanation of food waste. In fact, at higher loading rates, pretreatment can be detrimental to methane production due to increased acidification. These results indicated that in the BMP assays, methanogenesis was the rate-limiting step rather than hydrolysis. This is elucidated

when comparing the solubilization assays to the BMP assays. Within 24 h using a microbial inoculum, over 50% of pretreated food waste is solubilized; however in the reduced-loading-rate BMP assay, 4 days are required for 50% COD removal through methanogenesis. Increasing the methanogenic rate of a digester is, therefore, necessary in order to accommodate the increased solubilization of pretreated food waste through a greater metabolic flux of acidogenic products. Using a digester with a high methanogenic rate, such as a fixed-film reactor, can facilitate optimum anaerobic digestion of food waste. The retained microbial biomass in a fixed-film digester has a much greater methanogenic population, which can more quickly metabolize organic acids into methane. In a fully optimized system, methanogenesis would consume organics acids as they are produced. This would also allow the digester to operate at a higher loading rate because organic acids would not accumulate and cause acidification, as they did at moderate loading rates in the BMP assay. Experimentation with high-rate anaerobic digestion is necessary to verify the hypothesis that the increased solubilization from practical food waste pretreatment can result in enhanced biomethanation kinetics.

CHAPTER 5
CONCLUSIONS

The anaerobic digestion of food waste represents an opportunity for the diversion of food waste from landfills for the production of renewable energy and biofertilizer. Currently, food waste is not anaerobically digested as a sole feedstock in any commercial-scale anaerobic digestion facility. One limitation is the rate of hydrolysis of food waste, which is considered the slowest step in the anaerobic digestion of solid material. Hydrolysis in anaerobic digestion is largely facilitated by extracellular hydrolytic enzymes produced by the microbial consortia. These extracellular enzymes function through surface-area contact with the particulate substrate. Food waste, due to its high particulate nature, has a low surface area to volume ratio which impedes the rate of hydrolysis of the material. Therefore, by increasing the surface area and enhancing substrate availability for hydrolytic enzymes, the solubilization rate of food waste can be increased. Through increasing the surface area of food waste, tissue disruption and cell rupture occur, which releases endogenous soluble organic material and thereby enhances solubilization. Increased solubilization can increase the overall rate of food waste digestion by overcoming the rate-limitation of hydrolysis. To increase the solubilization rate of food waste, several methods of food waste pretreatment have been reported in the literature and have shown a positive correlation between pretreatment and increased solubilization and biomethanation kinetics. However, as these studies are at laboratory-scale, the pretreatment methods employed were not practical for commercial-scale digestion. Therefore, the current study examined the effects on anaerobic digestion kinetics of practical pretreatment methods (food disposer and meat grinder) that could be employed commercially. The two primary objectives of this study were to examine the impacts of pretreatment on the 1) solubilization kinetics and 2) biomethanation kinetics of food waste.

Solubilization Kinetics

A series of solubilization assays were conducted comparing the solubilization rates of pretreated and intact food waste. These experiments demonstrated that pretreatment has two principal impacts on the solubilization of food waste. First, endogenous SCOD was released immediately through pretreatment, while endogenous SCOD was released at a first-order kinetic rate of 0.36 h^{-1} from intact food waste. Second, pretreatment increased the rate of hydrolysis of food waste through increasing the substrate availability to hydrolytic enzymes. Using a commercial enzyme, the first-order kinetic rate constant were 0.84 to 1.19 h^{-1} for pretreated food waste hydrolysis and 0.25 h^{-1} for intact food waste hydrolysis. By 8 h pretreated food waste was 60% solubilized (28% through endogenous SCOD release and 32% through enzymatic hydrolysis). Intact food waste was 49% solubilized (21% though endogenous SCOD release and 28% through enzyme hydrolysis) in 8 h. A microbial inoculum was used in the solubilization assay as a surrogate for the hydrolytic microbial consortium in an anaerobic digester. Within 24 h incubation with the inoculum, 52% and 37%, respectively, of pretreated and intact food waste was solubilized. The solubilization kinetics using the inoculum also appeared to be biphasic with a second kinetic curve at 6 h. The biphasic kinetics may be due to an adaptation period required for the microbial inoculum to adapt to the fresh food waste in the assay. In both the initial and secondary hydrolysis phases, pretreated food waste showed a greater extent of enzymatic hydrolysis. This assay indicated that pretreated food waste was nearly as solubilized in 24 h with a microbial inoculum, as in 8 h with excess commercial hydrolytic enzymes. In an active, continuously-fed anaerobic digester, microbial enzymatic hydrolysis would not have the adaptation period observed in the assay and would show more rapid hydrolysis kinetics with the effect that hydrolytic enzymes produced by the microbial consortia could sufficiently solubilize pretreated food waste without the addition of expensive, commercial enzymes. The

solubilization assays in the present study indicated that pretreated food waste, using low-tech, practical methods, is a readily soluble substrate. The results of the assays support the hypothesis that practical, mechanical pretreatment can enhance food waste solubilization. Enhanced solubilization was accomplished through the disruption of tissue and rupture of cells, which both released endogenous soluble material and exposed surface area for hydrolytic enzymes.

Biomethanation Kinetics

Two biochemical methane potential (BMP) assays were conducted to compare the biomethanation kinetics of pretreated food waste with intact food waste. The first assay was conducted at a moderate loading rate of 3.5 g COD/L and the second at a reduced loading rate of 2 g COD/L. Both assays showed similar first order rate constants for pretreated and intact food waste: 0.12 d^{-1}in the first BMP and 0.20 d^{-1} in the second BMP. Intact and pretreated food waste at the reduced loading rate and intact food waste at the moderate loading rate showed similar 30 d extents of methane production at approximately 320 mL CH_4/g COD at STP or 91% COD removal. Pretreated food waste at the moderate rate, however, showed reduced 30 d methane production at 295 mL CH_4/g COD at STP or 83% COD removal. The reduced methanogenic kinetic rates at the moderate loading rate were due to acidification that overwhelmed the methanogenic population in the BMP assay. This was measured through conducting a simulated BMP assay at 2, 4, and 8 g COD/L from which samples were taken throughout 20 d for pH measurements. At the moderate loading rate, pH quickly dropped to below 6.5 within the first 3 days and was depressed below neutral for the first 10 d. Pretreated food waste at the moderate loading rate showed a greater pH depression, which correlates to the reduced 30 d methane yield from pretreated food waste in the first BMP assay. Therefore, it is concluded that in the BMP assays, methanogenesis was the rate-limiting step and not hydrolysis, and at a higher loading rate, increased solubilization and acidogenesis can inhibit

methanogenesis though acidification. Using a high-rate anaerobic digester, such as a fixed-film reactor, which contains a higher methanogenic population via retained microbial biomass, is necessary to accommodate the increased solubilization of pretreated food waste. Further experimentation is necessary to study the effects of using high-rate digestion on food waste pretreated using practical pretreatment methods.

Practical Implication of Research

One of the primary goals of this study was to study the impact of pretreatment on the anaerobic digestion of food waste without using expensive, impractical pretreatment methods that have been reported in the literature. Rather, the pretreatment methods studied herein employed equipment that is currently available throughout the community where food waste is generated. The use of such pretreatment equipment facilitates the commercialization of food waste digestion since the results found with these methods could be replicated on pilot- or commercial-scale digesters. In addition, an ethical argument could be made for the use of practical pretreatment methods, as the access to food waste digestion should not be limited by access to expensive or proprietary pretreatment methods.

To take full advantage of the increased solubilization kinetics of pretreated food waste, a high-rate anaerobic digester is ideal. A fixed-film digester with retained microbial biomass has a much higher concentration of methanogens, and hence a much higher methanogenic rate. Because the solubilization of pretreated food waste is so rapid, high methanogen populations are required to quickly convert the soluble organic material to methane. The increased solubilization of pretreated food waste also increases the acidogenic rate; therefore, a high methanogenic population that can rapidly consume the acids is necessary to reduce the potential for acidification in the digester. By coupling the benefits of practical pretreatment of food waste with high-rate anaerobic digesters, commercial-scale food waste digestion can become a reality.

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BIOGRAPHICAL SKETCH

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